MORPHOLOGICAL AND SUBMICROSCOPIC CHARACTERISTICS OF THE PRIMARY IMMUNOLOGIC RESPONSE AFTER DESTRUCTION OF THE POSTERIOR HYPOTHALAMIC NUCLEI

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The course of the primary immunologic response after unilateral destruction of the posterior hypothalamic nuclei is atypical, antibody synthesis is depressed, antibodies can be detected later, and the number of antibody-forming cells is reduced. Electron-microscopic investigations revealed a decrease in the functional activity of the plasma cells, a relative predominance of young undifferentiated cells of the plasma-cell series, and a decrease in the number of mature differentiated forms, evidently reflecting the reduced rate of differentiation of immunocompetent cells.

KEY WORDS: posterior hypothalamic nuclei; immunologic response; plasma cells.

The regulation of antibody formation by the hypothalamic–pituitary–adrenal system is a topic for wide discussion at the present time. The hypothalamus plays a special role in this complex mechanism of central regulation [1, 3-6, 8, 9, 14-16, 23].

Although the dynamics of the specific and nonspecific factors of immunity following injection of an antigen into animals with destroyed hypothalamic nuclei has been studied in some detail [7, 12, 13], the morphological substratum of the immune responses at the electron-microscopic level has not yet been investigated from this point of view.

This paper describes a study of morphological changes in the lymphoid tissue in the course of the immunologic response in animals with an intact brain and after unilateral "blocking" of the posterior hypothalamic nuclei.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred albino rats weighing 180-200 g. Unilateral destruction of the brain structures was carried out by the method adopted at the Central Research Laboratory of Rostov Medical Institute [16], with subsequent histological verification of the site of coagulation.

The animals were immunized 6-7 days after the operation by a single injection of a suspension (0.15 ml) of 30% sheep's red cells into the plantar surface of the hind limbs, and they were killed 2, 5, 7, 10, 15, and 20 days after immunization. The number of antibody-forming cells was determined by the method of Jerne and Nordin [20] in the modification of Nikolaev et al. [10] and calculated per million living nucleated cells. Serum antibodies (in log units) were determined by the usual methods.

Lymph glands of intact rats and of rats whose posterior hypothalamic nuclei were destroyed were subjected to electron-microscopic analysis on the 4th and 5th days after immunization. The pieces of tis-

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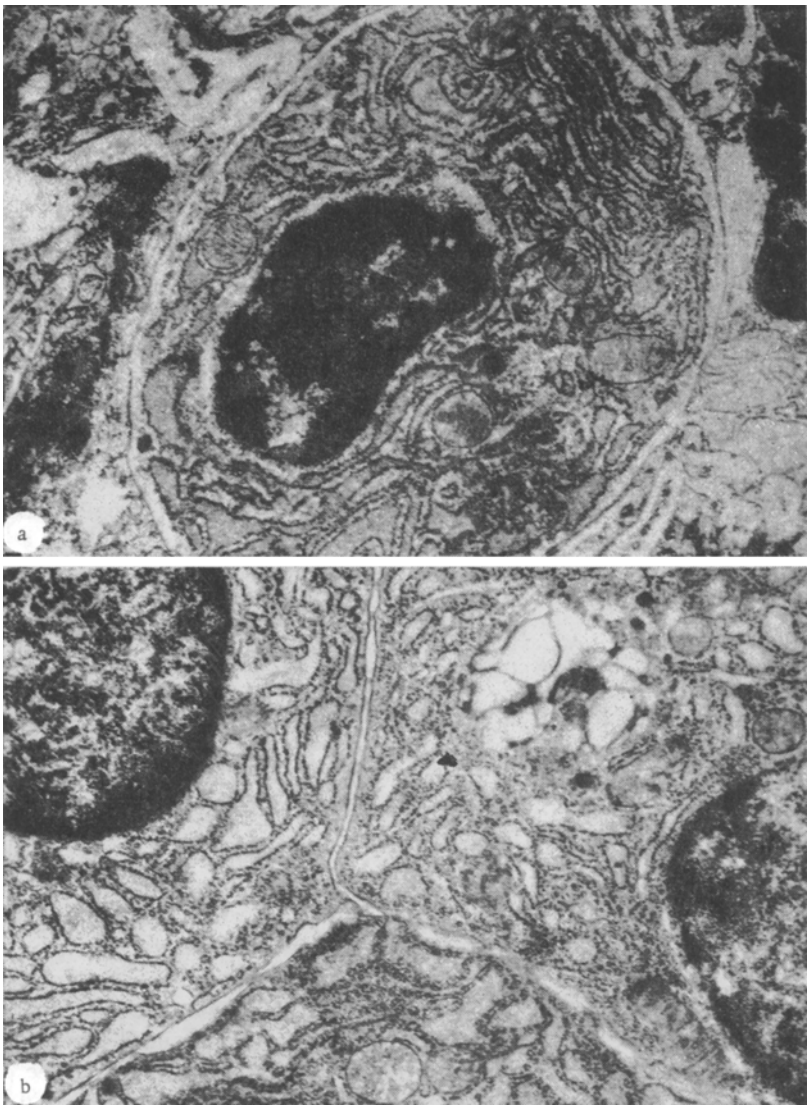

Fig. 1. Lymph gland: a) mature plasma cell on 4th day of immunization, 10,000×; b) group of mature plasma cells, 15,000×.

sue were fixed in 1% OsO_4 solution, dehydrated in alcohols and acetone, and embedded in Epon. Ultrathin sections were examined in the JEM-7 electron microscope.

Statistical analysis of the results was carried out by the method of indirect differences.

EXPERIMENTAL RESULTS

The number of plaques in the lymphoid tissue of the unimmunized rats did not exceed 0.2 per million cells in the lymph glands and 6.0 in the spleen.

The number of antibody-forming cells 2 days after injection of the antigen was significantly increased and reached a maximum on the 5th day (Table 1). The hemolysin-producing cells were eliminated just as quickly. On the 7th day after immunization the number of plaques fell sharply, evidently indicating regression of the process and considerable mortality among the antibody-forming cells [17, 18, 22].

On the 10th day of observation a tendency was again observed for the number of antibody-forming cells to rise, but later their number fell without returning to the initial level before the end of the investigation. Even though the dynamics of accumulation of antibody-forming cells was identical in the lymph glands and spleen, the intensity of this response was much higher in the regional lymph glands.

Parallel with antibody-forming cells, the humoral antibodies also were determined. Hemolysins appeared on the 2nd day after immunization and reached their maximum on the 5th-7th day (3.3 ± 0.06; 3.2 ± 0.03).

TABLE 1. Number of Antibody-Forming Cells in Tissues of Lymph Glands and Spleen and Number of Antibodies in Serum during Immunologic Response in Animals with Intact Brain (I) and after Destruction of Posterior Hypothalmic Nuclei (II)

Period of observation	Antibody-forming cells				Hemolysins		Hemagglutinins	
	lymph glands		spleen					
	I	II	I	II	I	II	I	II
Initially	0,19±0,14	—	5,5±1,1	4,8±0,57	—	—	—	—
2nd day	1,25±0,1	0,46±0,03	37,5±7,9	16,1±0,6	0,7±0,045	—	0,2±0,07	—
P		<0,001		<0,01				
5th day	2006±61,8	1088±62,0	489±10,5	129,0±13,8	3,3±0,06	2,2±0,16	1,5±0,13	1,3±0,16
P		<0,001		<0,001		<0,001		>0,1
7th day	69,0±10,6	22,8±5,3	13,0±2,9	15,2±2,6	3,2±0,03	2,2±0,23	1,8±0,09	1,2±0,21
P		<0,01		>0,1		<0,001		>0,1
10th day	92,0±16,4	4,3±1,0	50,0±10,5	5,6±0,9	2,6±0,17	1,9±0,2	2,6±0,21	2,1±0,15
P		<0,001		<0,01		<0,01		>0,1
15th day	16,0±1,6	17,6±4,08	47,6±10,9	12,7±1,8	1,9±0,23	1,7±0,21	3,1±0,07	1,3±0,26
P		>0,1		<0,01		>0,1		<0,01
20th day	5,8±1,0	21,1±4,8	20,9±3,7	4,9±1,02	1,7±0,16	0,7±0,34	2,4±0,18	1,3±0,27
P		<0,01		<0,01		<0,01		<0,01

Note: —) negative response.

Later their titer fell gradually and it remained at a fairly high level until the end of the investigation. Determination of the hemagglutinins revealed a somewhat different picture. They were found, as also were hemolysins, in very low dilutions of serum on the 2nd day after injection of the antigen, and they did not reach their maximal titers until the end of the 2nd week (3.1 ± 0.07), after which the titers remained high until the end of the experiment (Table 1).

These results provided a basis for the study of the possible effect of the functional state of the posterior hypothalamic nuclei on production of the primary immunologic response.

Determination of the antibody-forming ability of rats after preliminary destruction of the posterior hypothalamic nuclei revealed delay and depression of antibody formation.

At all periods of the investigation the hemolysin titers were significantly lower than in the animals of the control series, although the dynamics of hemolysin accumulation was identical in the animals of the two series. The hemagglutinin titers during the first few days after immunization did not differ significantly from the control, but later (15th and 20th days) the difference was considerable and significant ($P < 0.01$; Table 1).

After destruction of the posterior hypothalamic nuclei the number of antibody-forming cells in the lymph glands and spleen of the animals was significantly less at all times of investigation than in the control series (Table 1). However, in unimmunized animals after destruction of the posterior hypothalamic nuclei the number of plaques in the spleen did not differ statistically significantly from the number in the control animals; antibody-forming cells were detected in the lymph glands in only 1 of the 6 rats. Changes also took place in the dynamics of accumulation on antibody-forming cells. After reaching a maximum on the 5th day the number of cells fell sharply on the 7th day and even more considerably until the 10th day; the increase in the number of cells on the 10th day of immunization, characteristic of the animals of the control series, was not observed. Evidently destruction of the posterior hypothalamic nuclei disturbed the rhythm of commencing differentiation of the precursor cells.

By the end of the 3rd week of the experiment the number of cells in the lymph glands continued to increase (20.9 ± 3.7), whereas in the spleen it returned to its initial level (4.9 ± 1.02).

During immunization of animals after destruction of the posterior hypothalamic nuclei the primary immunologic response followed an atypical course and the reduced number of antibody-forming cells led to a decrease in the synthesis of specific antibodies. The possibility of a reduced antibody-synthesizing function of the antibody producer cells likewise cannot be ruled out.

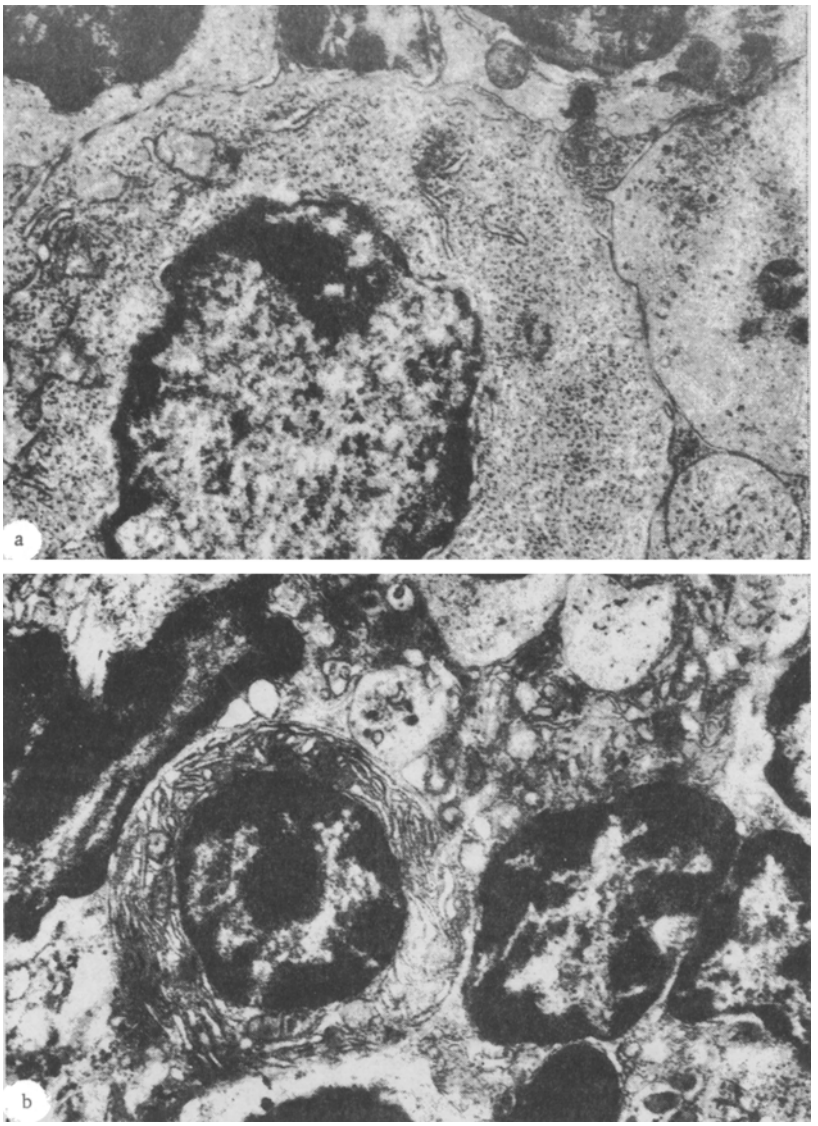

Fig. 2. Lymph gland on 5th day after immunization after destruction of posterior thalamic nuclei: a) blast cell, 10,000×; b) lymphoplasmacyte, 5000 ×.

To study the causes of this effect the immunocompetent cells of the regional lymph glands were investigated by electron microscopy.

The ultrastructure of lymph gland cells has been well studied [2, 11, 9], and its description in intact animals can therefore be omitted.

During immunization definite hyperplasia of cells of the plasma-cell series in different stages of differentiation was found. Their maturation was accompanied by development of elements of the rough endoplasmic reticulum, which in the mature form occupied nearly all the cytoplasm and consisted of dilated cavities and cisterns, filled with material of average electron density (Fig. 1a). The number of polysomes and free ribosomes fell sharply; the latter were fixed on membranes of the endoplastic reticulum. The mitochondria resembled large particles with a translucent matrix. The Golgi complex was hypertrophied, displacing the nucleus to the periphery, and around it there was an increased number of spherical electron-dense granules, evidently secretory in character. Their formation also was observed in the dilated cavities of the ergastoplasm. The number of activated lymphocytes was increased.

On the 5th day after injection of the antigen, cells of the plasma-cell series consisted mainly of adult forms (Fig. 1b). Cells in a state of high functional activity, containing Russell's bodies in their cytoplasm, and disintegrated cells were found at the same time. The plasma membranes of the distintegrated cells

had lost their structural organization, the ergastoplasm and other organelles were disorganized, and the contents of the cisterns were reduced or had disappeared.

Submicroscopic investigation of the lymph glands of animals after destruction of the posterior hypothalamic nuclei showed that the operation itself did not affect the populations of cells or their ultrastructure. Immunization after this procedure characteristically did not give rise to proliferation and transformation of such high intensity as in the animals of the control group.

On the 5th day after immunization the predominant cells of the plasma-cell series were young undifferentiated forms of the blast type (Fig. 2a) and there were very few immature plasma cells or transitional forms. Mature plasma cells also were few in number and showed signs of weak functional activity. Meanwhile there was an increased number of cells with a central nucleus, whose cytoplasm was filled with intact cisterns and with tubules of the endoplasmic reticulum (Fig. 2b). In their description they resembled lymphoplasmacytes [21].

To sum up the results of electron-microscopic analysis, it can be concluded that in animals immunized after coagulation of the posterior hypothalamic nuclei the course of differentiation of the young cells of the plasma-cell series is evidently disturbed, with a consequent reduction in the number of differentiated forms, the principal producers of antibodies.

These investigations, conducted at the microscopic and submicroscopic levels, have thus shed light on the morphological changes responsible for the weakening of the primary immunologic response following "blocking" of the posterior hypothalamic structures.

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