MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION OF THE ORGANS OF IMMUNOGENESIS DURING INTRACEREBRAL GROWTH OF TRANSPLANTED GLIOBLASTOMA MULTIFORME IN ALBINO RATS

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Morphological changes in the organs of immunogenesis (thymus, lymph glands, spleen) were studied and the RNA content of these organs determined biochemically in 92 rats during growth of a transplanted intracerebral glioblastoma multiforme (strain No. 101/12). The response of the lymphoreticular organs was shown to depend on the stage of the disease. On the 6th day after transplantation of the tumor, for instance, activation of the reticuloendothelial part of the thymus, hyperplasia of the lymphoid follicles in the lymph glands, and activation of nucleic acid synthesis were observed, whereas in animals with a developing tumor death of the lymphoic elements and a decrease in RNA sysnthesis were found. Morphological investigation of the spleen showed a gradual reduction in its cell population.

KEY WORDS: transplantable tumors; thymus; lymph glands; spleen.

Much experimental evidence of differences in the antigenic structure of normal and tumor tissues has recently been obtained [1, 2]. Morphological changes in the lymphoreticular organs are among the inevitable consequences of interaction of the organ-specific antigen–antibody type. Each of these organs (thymus, lymph glands, and spleen) performs its own particular function. Whereas the thymus plays a basic role in immunologic surveillance of the tumor, the spleen and lymph glands are directly concerned in the protective responses of humoral and tissue character [3]. The RNA content in these organs is an indicator of antibody production, for antibody synthesis is linked with the accumulation of RNA [4].

The object of this investigation was to make a morphological study of the organs of immunogenesis during the development of a transplantable tumor in the brain and to determine their RNA content biochemically.

EXPERIMENTAL METHOD

Experiments were carried out on 92 noninbred infantile female albino rats (weight 50-70 g) into which a glioblastoma multiforme (strain No. 101/12) was transplanted intracerebrally. The animals were killed on the 11th day after transplantation of the tumor, and at a time of appearance of distinct clinical evidence of the disease (15.7 ± 1.06 days after the operation). The control group consisted of intact animals killed simultaneously with the experimental group. The thymus, lymph glands, and spleen were taken for morphological examination, fixed in Carnoy's fluid, embedded in paraffin wax, and stained with hematoxylin–eosin in Brachet's and the PAS reactions were carried out. RNA was determined quantitatively by the method of Schmidt and Thannhauser [5].

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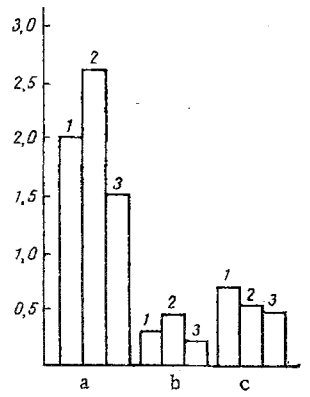

Fig. 1. Total RNA content in thymus, lymph glands, and spleen. Ordinate, RNA content (in mg/g protein); abscissa; a) thymus; b) lymph glands; c) spleen. 1) Intact animals; 2) animals on 6th day after transplantation of tumor; 3) rats with developing brain tumor.

EXPERIMENTAL RESULTS

Histological investigation of the thymus glands of rats killed on the 6th day after transplantation of the tumor revealed preservation of the boundaries between the layers; both layers were reasonably wide. Small foci of depopulation were found in the cortex, together with groups of pyroninophilic cells, whereas in the medulla the cells of the reticuloendothelium were juicy and pyroninophilic on staining by Brachet's reaction. The Hassall's corpuscles were large, some of them contained ingested particles, and the PAS reaction revealed a small quantity of PAS-positive material in them. Both cortex and medulla contained numerous reticuloendothelial cells with mitotic figures.

In the animals with a developing primary tumor, areas containing groups of confluent hyperchromic thymocytes appeared in the cortex of the thymus. Together with these changes, many macrophages with phagocytosed cells and particles of fragmented cells, giving a positive Brachet's reaction, were seen in the cortex. The Hassall's corpuscles varied in size and number, but they were much smaller than in rats killed on the 6th day after the operation. A few mature and immature plasma cells were observed in the cortex and medulla. The immature cells were often located in the perivascular connective tissue. Reticuloendothelial cells with mitotic figures were found in the cortex only.

Some degree of activation of the reticuloendothelial component was thus found in the thymus of rats killed on the 6th day after the operation, but if a developing tumor was present, degenerative changes in the thymocytes and a macrophagal reaction occurred.

In the parallel biochemical investigation of the RNA content in the thymus, on the 6th day after the operation the RNA content was higher than normal, but in the rats with a developing tumor it was lower than in the intact animals (Fig. 1).

Morphological investigation of the lymph glands of rats killed on the 6th day after the operation showed a disturbance of the architectonics of the lymph glands. The cortex consisted of clusters of lymphocytes, with solitary pyroninophilic cells distributed among them. Follicle-like structures were scattered throughout the area of the lymph gland (Fig. 2). Their pale centers contained reticulum cells with mitotic figures, macrophages, and also cells with pyroninophilic cytoplasm and a palely-stained nucleus (immunoblasts). The medullary cords were relatively wide and contained mature and immature plasma cells. The sinuses were dilated and their endothelium was swollen.

In the animals with a developing tumor the structure of the lymph glands was grossly disturbed. The cortex consisted of a continuous sheet of large lymphocytes and reticuloendothelial cells. Small follicle-like formations containing pyroninophilic cells with a large, pale nucleus, were revealed only by Brachet's reaction. The medullary cords were narrow, with grossly dilated sinuses, the lumen of which was packed with desquamated endothelial cells. A few plasma cells were present in the medullary cords.

On the 6th day after transplantation of the tumor into the brain, structural disturbances were found in the lymph glands, with hyperplasia of the pale centers of the follicle-like formations and the appearance of numerous plasma cells; if a developing tumor was present, the structural disturbances were severe, very few lymphocytes were present, and the number of pyroninophilic cells was reduced.

Biochemical determination of the RNA content in the lymph glands on the 6th day after the operation showed an increase, but during development of the tumor its content was below normal (Fig. 1).

In the spleen, on the 6th day after the operation the malpighian bodies varied in size, their pale centers were not clearly distinguishable, and no pyroninophilic cells could be seen. The central arteries had swollen endothelium.

Some reduction in the density of the red pulp was observed. Lymphocytes in it were grouped in large clusters (Fig. 3a) and a few mature and immature plasma cells also were present, mainly beneath the capsule. A few megakaryoblasts also were present, their cytoplasm weakly pyroninophilic.

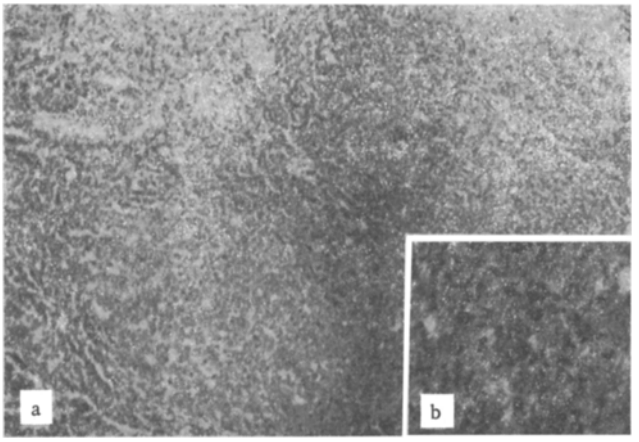

Fig. 2. Follicle-like structures in lymph gland of rat on 6th day after transplantation of tumor: a) general view of lymph gland, stained with methyl green-tyronine, 120 ×; b) area of pale center of follicle-like structure, stained with methyl green-tyronine, 500 ×.

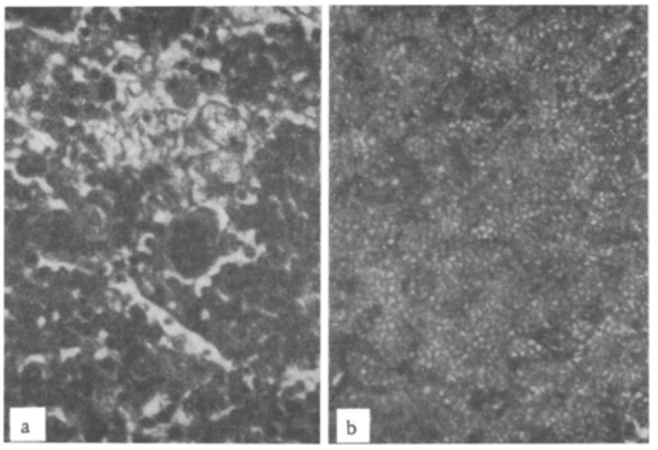

Fig. 3. Spleen of rats with transplanted tumor: a) lymphocytes in large clusters in red pulp of spleen on 6th day after transplantation of tumor; hematoxylin–eosin, 500 ×; b) marked decrease in number of lymphocytes in spleen, but numerous erythrocytes in it in rats with developing brain tumor; hematoxylin–eosin, 500 ×.

The malpighian bodies in the spleen of rats with a developing brain tumor were reduced in size and their pale centers were absent. The central arteries had swollen walls and pyconotic endothelium. The red pulp contained a few lymphocytes. The sinuses were grossly dilated and filled with erythrocytes. As a result of increased permeability of the vessels the red pulp thus resembled "lakes of blood" with tiny islands of lymphoid tissue (Fig. 3b). There were few or no megakaryoblasts.

The biochemical tests also confirmed a gradual decrease in the RNA content of the spleen during development of the brain tumor (Fig. 1).

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