DYNAMICS OF ADRENAL MORPHOLOGY AND FUNCTION IN IRRADIATED RECIPIENTS AFTER BONE MARROW TRANSPLANTATION

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Under the influence of irradiation profound disturbances of the morphological and functional properties of organs of the lympho-myeloid complex develop and are among the principal causes of death. Restoration of hematopoiesis, including lymphopoiesis, and of the histological structure of the hematopoietic and lymphoid organs can be achieved with the aid of native and frozen hematopoietic cells [1, 2, 5]. Research in these fields is being conducted on an intensive scale [6, 10, 12], but the study of the character of the changes and of restoration of the properties of the neuroendocrine organs in irradiated recipients, protected with bone marrow cells, has so far been extremely inadequate, and the results obtained have been sporadic and contradictory.

The aim of this investigation was to study the state of the adrenals in lethally irradiated recipients at different stages after transplantation of native and frozen hematopoietic cells.

EXPERIMENTAL METHOD

Experiments were carried out on 150 inbred male (CBA × C57BL)F_1 mice aged 2 months. The animals were divided into four groups: 1) lethally irradiated animals, 2) lethally irradiatated animals receiving an injection of native syngeneic bone marrow, 3) lethally irradiated animals receiving syngeneic frozen bone marrow, 4) control (intact animals). Irradiation was given on the RUM-17 apparatus. The conditions of irradiation were: dose rate 39.5 R/min, 200 kV, current 10 mA, filter 0.5 mm Cu + 1 mm Al. Bone marrow was isolated from the mouse femur and frozen by the method in [11]. Syngeneic bone marrow from donor mice, both native and frozen, was injected intravenously in a dose of $1 \cdot 10^7$ cells/ml. The animals were irradiated and the bone marrow transplanted at the same time in the fall and winter from 1 a.m. to noon. The state of the adrenals was studied 3 h and 1, 3, 7, 10, 20, 30, 40, 60, and 90 days after irradiation and bone marrow transplantation. Three animals for the experimental groups and three animals for each control group were used at each time. The animals were weighed before decapitation. After removal, the adrenals were weighed; the right adrenal was then fixed in 10% neutral formalin for subsequent study of its histological structure, whereas the left adrenal was used for histochemical determination of lipids. Histological sections 4-6 μ thick were stained with hematoxylin and eosin, and frozen sections of the left adrenal were stained with Sudan III and Sudan Black B by Lyson's method [4]. The thickness of the cortical layer of the adrenals was determined on histological sections by application of a grid with equidistant points [3]. The degree of sudanophilia was estimated by the use of the histochemical coefficient of Astaldi and Verga [13]. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The weight of the adrenals of the control animals varied within very narrow limits. In histological sections the connective-tissue capsule of the adrenals, consisting of two or three layers of fusiform cells, was clearly defined. Immediately under the capsule lay the zona glomerulosa of the cortex, in which the cells were arranged in small groups. Next

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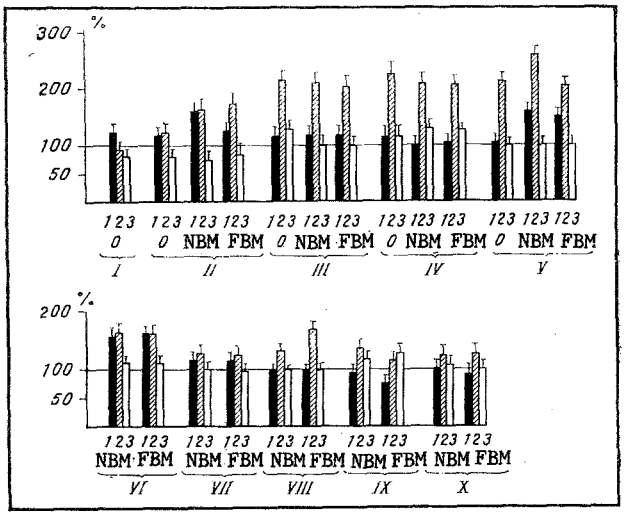

Fig. 1. Changes in morphological and functional parameters of adrenals in irradiated recipients after transplantation of bone marrow (in % of control). 1) Relative weight of adrenals; 2) thickness of adrenal cortex; 3) degree of sudanophilia; 0) irradiation, NBM) transplantation of native bone marrow, FBM) transplantation of frozen bone marrow: I) 3 h after irradiation, II-X) times after irradiation and bone marrow transplantation (in days) were 1, 3, 7, 10, 20, 30, 40, 60, and 90, respectively.

could be distinguished the wide layer of the zona fasciculata, whose cells formed straight bands running radially, with straight capillaries passing between them. Between the medulla and the zona fasciculata lay the comparatively thin layer of the zona reticularis of the adrenal cortex, whose cells formed irregular bands. The parenchymatous cells of the zona glomerulosa closely resembled cells of cylindrical epithelium in their morphology. The cytoplasm of the spongiocytes was vacuolated due to their high content of lipids. In the zona reticularis cells with hypochromic nuclei and small cells with hyperchromic nuclei and homogeneous basophilic cytoplasm could be distinguished. Cells of the medullary layer were large and polymorphic in shape, and less intensely stained than cells of the cortical layer. The sinuses of the medullary layer were filled with blood cells.

In the animals of group 1 histological changes were observed as early as 3 h after irradiation. Swelling of the collagen fibers of the capsule and loosening of their arrangement took place; the cortical layer was enlarged and the organs were congested. Degenerative and destructive changes in the parenchymatous cells gradually increased in severity in both zona glomerulosa and zona fasciculata, in the form of pycnosis and lysis of the cell nuclei and indistinctness of the cell boundaries. Very intensive vacuolation of the cell cytoplasm was observed, especially in the zona fasciculata. The histological structure of the medulla was unchanged. The character of the histological and histochemical changes noted remained unchanged throughout the period of observation until death of the animals. As will be clear from Fig. 1, the relative weight of the adrenals in the animals of group 1 during the first few hours after irradiation exceeded the weight of the adrenals of the control animals (by 17%). Morphometric analysis of the histological preparations shows that 3 h after irradiation the area of the adrenal cortex was reduced, but not significantly (by 7% compared with the control), but from the 1st through the 7th days it increased sharply in size (by 37% compared with the control). By the time of death of the animal the thickness of the cortex was very slightly reduced and exceeded the control appreciably. The lipid content in the interval between 3 and 24 h, especially in cells of the zona fasciculata, was 16% below the control level, but starting with the 3rd day it rose and then fell sharply again toward the beginning of mass death of the animals (10th day).

In the animals of groups 2 and 3, during the first day after irradiation and bone marrow transplantation moderate loosening of the collagen fibers of the adrenal capsule was observed. Congestion of the organ and widening of the zona fasciculata on account of hypertrophy of its

cells were observed. Destructive changes in cells of the zona glomerulosa and the combined zona fasciculata and zona reticularis were less marked in the animals of these groups than in the lethally irradiated animals unprotected by bone marrow. On the 10th-20th day hypertrophy of the cortical cells still remained and single cells with pycnotic and lysed nuclei were seen. The histological structure of the adrenal cortex in the animals of these groups returned to normal after 30-40 days. Depending on the stage of the post-transplantation period changes were found in the weight of the adrenals in these groups of animals. For instance, the weight of the adrenals rose sharply above the control value as early as 24 h after transplantation of both native and frozen bone marrow (by 54 and 44%, respectively). The time course of the weight of the recipients' adrenals was wavelike in character. Peaks of maximal values in the animals of group 2 preceded the corresponding values of the weight of the adrenals in the animals of group 3, in which the weight of the glands decreased starting with the 40th day. The area of the adrenal cortex rose sharply in the animals of both experimental groups during the first day after bone marrow transplantation (by 62 and 66%, respectively) compared with the control, and continued to rise on the following days, to reach a maximum after 10 days. The cortex then decreased in thickness, but still remained thicker than the control (by 23 and 37%, respectively), even on the 90th day. Changes in the lipid content in the adrenals also were wavelike in character, and their content in the cortical cells of the recipients fell by 27-30% on the 1st day, but rose until the 3rd day to reach the control value. The first maximum of the lipid content was observed on the 7th day, and by the 10th day it was close to the control level. The second maximum of lipid accumulation occurred on the 20th day, and in the interval between the 30th and 40th days their content fell. Starting with the 60th day, the degree of sudanophilia increased again.

It can thus be concluded on the basis of these results that during lethal irradiation of male mice functional and morphological changes in the adrenals, specifically in the adrenal cortex, are nonspecific in character and are similar to those in the stress response, namely an increase in weight of the adrenals, marked congestion of the glands, and widening of the adrenal cortex on account of the zona fasciculata and zona reticularis. With an increase in the period of time after irradiation the character and activity of the adrenal responses are probably determined not so much by the direct action of radiation on the endocrine gland as by the action of various breakdown products accumulating in the organs and systems of the body in response to irradiation. Similar results also were obtained by other workers [7-10]. Bone marrow transplantation into lethally irradiated recipients, depending on the stage of the post-transplantation period, leads to wavelike changes in weight of the glands, in the thickness of the adrenal cortex, and in the lipid content in the spongiocytes, evidence that the processes taking place in the adrenals of the animals of these groups follow a definite time course.

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