MORPHOLOGICAL CHANGES IN THE THYMUS AND SPLEEN OF NEWBORN MICE EXPOSED TO LOCAL IRRADIATION WITH A MASSIVE DOSE OF X-RAYS

(UDC 617-001.26-053.31-092.9-036.8-07:[616.438+616.411]-091-07)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 6, pp. 103-107, June, 1966
Original article submitted December 14, 1964

It became known from Miller's work [8] that the removal of the thymus from mice during the early days of life leads to an acute disturbance in the formation of the normal immunological status of the organism. The latter concerns antibody formation and transferred immunity [2, 6]. Besides this, serious changes take place in the lymphoid tissue of thymectomized animals; small lymphocytes do not develop in it [4, 9].

The radiosensitivity of the lymphocytes of the thymus and its reticular and epithelial elements are not identical. The lymphocytes are highly sensitive to radiation but the epithelial cells are comparatively resistant. It has been established that the lymphocytes of the cortical layer are highly sensitive to radiation in comparison with the lymphocytes of the medullary layer [1, 11].

Local exposure of areas of the thymus of adult mice to x-rays in doses of 3000 r led to almost complete disappearance of lymphoid cells in the organ itself and to a decrease in the circulation of lymphocytes in the peripheral blood with a subsequent restoration of their original number [5].

In the present research work local exposure of the thymus of newborn mice was carried out with high doses of x-rays in order to try to eliminate lymphoid tissue only, leaving the basic tissue elements of its stroma intact (in contrast to surgical thymectomy of the newborn when the whole thymus is removed). It was proposed to follow the regeneration of the thymus under these conditions and the changes in the lymphoid tissue in another part of the animal, for example, the spleen.

EXPERIMENTAL METHOD

Work was carried out on both sexes of newborn mice of strain C57BL at an age up to 24 h after birth. Exposures were given in the RUM-3 apparatus at a dose of 3000 r through filters of 0.5 Cu and 1.0 Al, the dosage rate being 116 r/min at a distance of 14 cm from the x-ray source. The mice were placed in a lead box and secured, back downwards, to a cork base. The box was covered with a lead plate, 8 mm thick, having an opening the area of which slightly exceeded that of the thymus. The animals were killed 2-167 days after exposure. The thymus and spleen were fixed in alcohol-formalin. The serial paraffin sections were stained in hematoxylin-eosin by the polysaccharide SHIK-method and in methyl green-pyronine.

EXPERIMENTAL RESULTS

In normal, nonirradiated animals it is possible to distinguish the cortical and medullary layers already on the 3rd day. Basically, the former consists of small lymphocytes, the larger ones being met with in the medulla. By the 15th day the thymus is well formed and clearly differentiated Hassall's corpuscles are present.

Two or three days after irradiation (see figure, a) there were many disintegrating lymphocytes in the thymus. They were located in the cortical and medullary layers, here and there forming small accumulations. Small lymphocytes were almost completely absent. In the peripheral layers of the cortex, and also in the medulla on a background of destroyed lymphatic tissue lay single large, pyroninephilic cells of a lymphoblastic type. Hassall's corpuscles with epithelial cells were prominent and contained polysaccharides in their cytoplasm.

In two animals killed on the 4th day, no substantial reduction of lymphoid tissue was noticed and the thymus contained only a larger number of large pyroninephilic cells of a lymphoblastic type.

On the 5th and 6th days the thymus was in a condition in which the lymphatic tissue was noticeably reduced but, nevertheless, the cortical and medullary layers were distinguishable. No definite fresh foci of degeneration or cell debris were observed. In the cortical layer, cells of a lymphoblastic type were met with and also large lymphocytes, among which numerous mitoses were seen (see figure, b).

Together with cells of a lymphoblastic type there was a large amount of diffusely scattered cell debris in the thymus on the 8th day, principally in the cortical layer. Here also, large centers of small degenerating lymphocytes were seen.

On the 10th-13th days, the lymphoid tissue of the thymus was in a state of almost total degeneration. The thymus was represented as a well-preserved epithelial stroma, on the background of which was cell debris in the form of drops (see figure, c). In one animal, lymphocytes were completely absent from the thymus and in their place were drops of chromatin on a background of epithelial tissue which was morphologically intact. In another animal a small number of lymphoblasts survived, principally in the cortical layer.

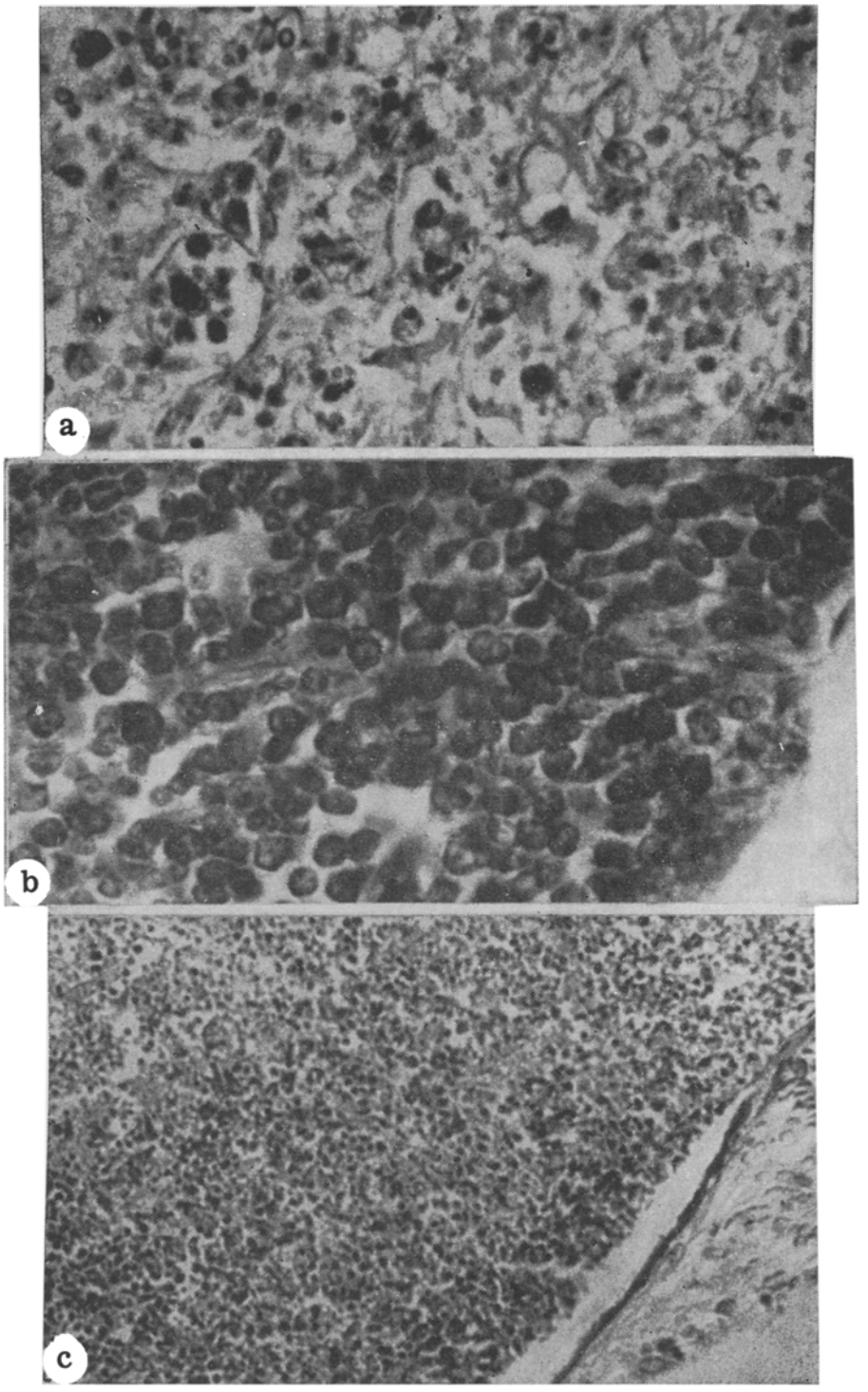
On the 16th-18th days, the lymphoid tissue of the thymus continued in an atrophied state but no cell debris was observed. In the cortical and medullary layers a large number of lymphoblasts and large lymphocytes were noticed, among which numerous mitoses were seen. The epithelial cells of the stroma were in a condition of active secretion. In one mouse, on the 16th day, a center of active myeloid blood formation was found in the thymus.

No plasma cells were observed in any of the preparations.

After 22-167 days the irradiated thymus was no different in structure from the normal thymus of an animal of corresponding age.

The spleen of nonirradiated, 3-day-old mice consists basically of myeloid, hematopoietic tissue. The lymphoid cells are small and do not form independent structures. The spleen of 3-day-old irradiated animals was of a similar construction. In nonirradiated, 8-day-old mice, the lymphoid structures were of a considerable size and located along the course of the vessels. In irradiated, 8-day-old mice, lymphoid cells were extremely rare in the spleen, isolated lymphoid structures were not noticed, and the organ was reminiscent of the spleen of 3-day-old animals. At the age of nine days the spleen of normal animals contained typical Malpighian bodies consisting of lymphoid tissue. Their peripheral zones were occupied by small lymphocytes. A small number of lymphoid cells were observed, in the spleen of 9- and 11-day-old irradiated mice, but independent lymphoid structures were absent. They appeared on the 13th day in irradiated animals in the form of lymphocyte accumulations along the course of the vessels. In a number of irradiated animals, 14-18 days old, the lymphoid tissue of the spleen was, as before, sharply reduced and lymphoid structure were absent. Malpighian bodies were discerned in other animals. In 22-day-old irradiated mice the spleen had the same structure as that of the controls.

Thus, local irradiation of the thymus of newborn mice with a dose of 3000 r led to a considerable change in the lymphoid tissue but caused no visible morphological disturbance in the epithelial stroma of the thymus. During the first days following irradiation, a sharp reduction in lymphoid tissue was observed while the epithelial stroma survived. After 4-8 days, regeneration of lymphoid tissue was seen, and an increase in the large proliferating lymphocytes and the lymphoblasts was noticed in the atrophied thymus. The first wave of degeneration which overran the more mature lymphoid cells in the thymus of newborn animals at the time of irradiation depended, evidently, on the direct action of the irradiation. The causes of the second wave of degeneration were not clear. It had a much more definite character as a total destruction of the lymphocytes was observed. It may be presumed that the second wave was connected with the attainment of the adult stages of differentiation by the propagating forms on which radiation damage suffered by their cell predecessors appeared. However, in spite of an almost complete necrosis of the lymphoid tissue of the thymus, substantial regeneration took place.



The thymus of the mouse irradiated with a dose of 3000 r in the first hours of life. a) Two days after irradiation. Destruction of lymphoid tissue with preservation of the epithelial stroma. Drops of debris in place of lymphocytes. Obj. 40X; b) six days after irradiation. Regeneration of lymphoid tissue. A great number of large pyroninephilic cells. Obj. 40X; c) eleven days after irradiation. Almost complete necrosis of the lymphocytes of the thymus. Obj. 24X.

Considering the data obtained during medical treatment of irradiated animals by labelled lymphoid cells, it may be assumed that the irradiated thymus is regenerated mainly by the multiplication and differentiation of lymphocytes coming from without and repopulating it [3]. Since irradiation was conducted within 24 h after birth, it must be presumed that the thymocytes had not yet repopulated the peripheral organs of the lymphoid system and, consequently, in this respect, our animals could be compared with those in which the thymus had been surgically removed on the first day of life. As regeneration of lymphoid tissue took place in our animals, it is evident that peripheral lymphocytes can become thymocytes, the forerunners of which are known not to be derived from cells originating in the thymus. Hence, it can be concluded that, compared with other lymphocytes in the organism, generation of lymphocytes present at the moment of birth possesses no unusual properties.

Distinct signs of the rudiments of lymphoid tissue were observed in the spleen of irradiated animals up to the 18th day after birth. This confirms that the function of the thymus, while stimulating the development of lymphoid tissue, was also actively connected with the spleen. The attainment of the normal structure by the nonirradiated spleen coincided in time with the regeneration of lymphoid tissue in the irradiated thymus.

Morphologically, there were no degenerative changes in the epithelium of the irradiated thymus, and it is not very probable that its function could be upset to any extent. However, judging from the rudiment of lymphoid tissue in the spleen, the epithelium by itself did not provide for those actions of lymphoid tissue which normally arise from the thymus. Moreover, the survival of the epithelium proved to be sufficient to re-establish the thymus itself and the peripheral lymphoid tissue. This distinguishes our results from those given by surgical thymectomy of newborn mice.

It may be presumed that the epithelium of the thymus provides local conditions for the histogenesis of thymocytes (lymphocytes) and that the effect on the maturation of lymphoid tissue in the whole of the organism proves to be not the epithelium itself, but that structure which arises from the association of the epithelium of the thymus with lymphocytes [7, 10]. One can only speculate on the nature of these effects. The question concerning the origin of the cells in the regeneration of thymocytes is unsolved. This pertains to regeneration of the thymus after irradiation and after transplantation. In the latter instance, data obtained from the labelling of chromosomes is contradictory and, thus, regeneration cannot be ascribed solely to repopulation by lymphocytes.

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