OSTEOGENIC POTENTIAL OF IRRADIATED BONE MARROW TRANSPLANTED IN DIFFUSION CHAMBERS

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Bone marrow from irradiated mice treated with injection of autologous marrow and from untreated mice was transplanted into diffusion chambers. On the 5th day after irradiation the marrow had no osteogenic potential. The osteogenic power of the irradiated marrow began to be restored on the 10th day after irradiation, and by the 15th day it was close to normal. The osteogenic potential of irradiated marrow correlates with the number of marrow cells contained in the femur.

When bone marrow is transplanted into diffusion chambers hematopoiesis stops, reticulum tissue proliferates, and after the 3rd day intensive osteogenesis begins [1]. A certain initial density of marrow cells in the chamber is essential for the development of osteogenesis [2]. It may be asked what category of cells in the heterogeneous population of marrow cells is responsible for the development of osteogenesis and, in particular, whether these cells are hematopoietic stem cells (or their progenies) or whether they constitute an independent cell line.

The object of the present investigation was to determine the osteogenic potential of irradiated bone marrow at various stages of its regeneration.

EXPERIMENTAL METHOD

Adult C57Bl and C3H mice were used. The bone marrow donors were irradiated in a dose of 750 R on a cobalt apparatus. The donors of one group received an intravenous injection of 10^7 homologous bone marrow cells 2-4 h after irradiation. The donors were sacrificed on the 5th, 10th, and 15th days after irradiation, the marrow from one femur was expelled by means of a syringe, and one piece of marrow was placed in each of a series of diffusion chambers [3]. The chambers were implanted intraperitoneally into animals of the same line. Marrow from the second femur was used to prepare a cell suspension in which the cell count and formula were determined. On the 15th-30th day the chambers were removed and dismantled and the filters were fixed in 96° alcohol in the cold. Gomori's test for alkaline phosphatase was carried out on the filters, and they were then counterstained with alumhematoxylin and examined as total preparations. Altogether 63 chambers were used.

EXPERIMENTAL RESULTS

The morphological pictures in the chambers with bone marrow explants from the treated and untreated mice did not differ appreciably. The contents of the chambers showed regular changes depending on the time after irradiation. After implantation of tissue taken on the 5th day after irradiation, large cells resembling fibroblasts were found in the chamber, where they did not form a continuous layer on the surface of the filter. Some of these cells were binuclear (Fig. 1, a). Besides fibroblast-like cells, numerous histiocytes containing granules of brown pigment in their cytoplasm, and degenerating cells were found in the chambers. Neither bone structures nor other phosphatase-positive structures were found.

In the chambers containing marrow transplanted 10 days after irradiation of the donors the amount of tissue was increased. It consisted of fibroblast-like cells covering the filters in a solid layer. These cells, rectangular in shape, had a large, long nucleus (sometimes 2-3 nuclei). Residues of dead spongy bone, introduced into the chamber at the time of explantation, were seen on some filters. The greater part of the

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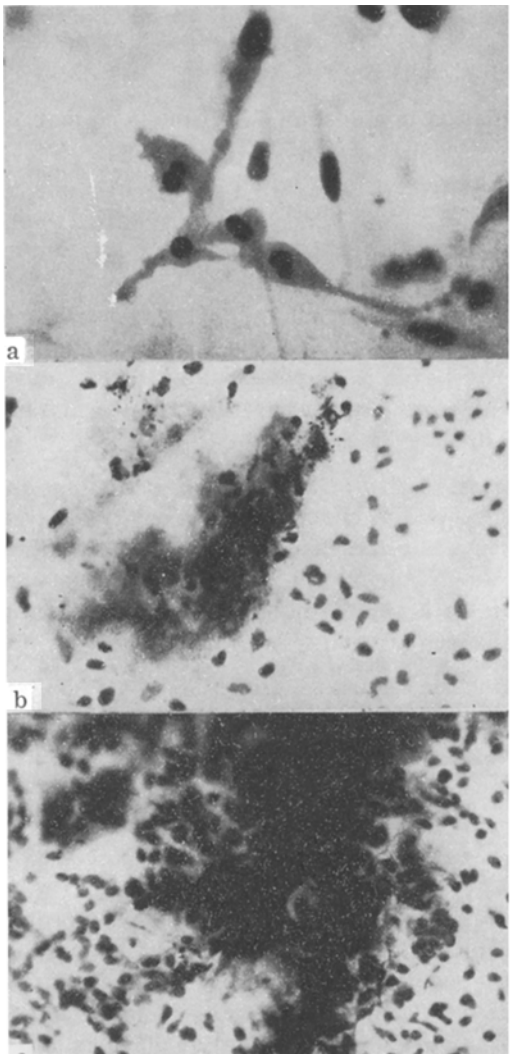

Fig. 1. Contents of diffusion chambers with transplanted marrow from mice treated after irradiation by injection of marrow cells. a) 5 days after irradiation: fibroblast-like cells, some of them binuclear, objective 40; b) 10 days after irradiation: typical focus of osteogenesis, objective 20; c) 15 days after irradiation: osteogenic focus, objective 40.

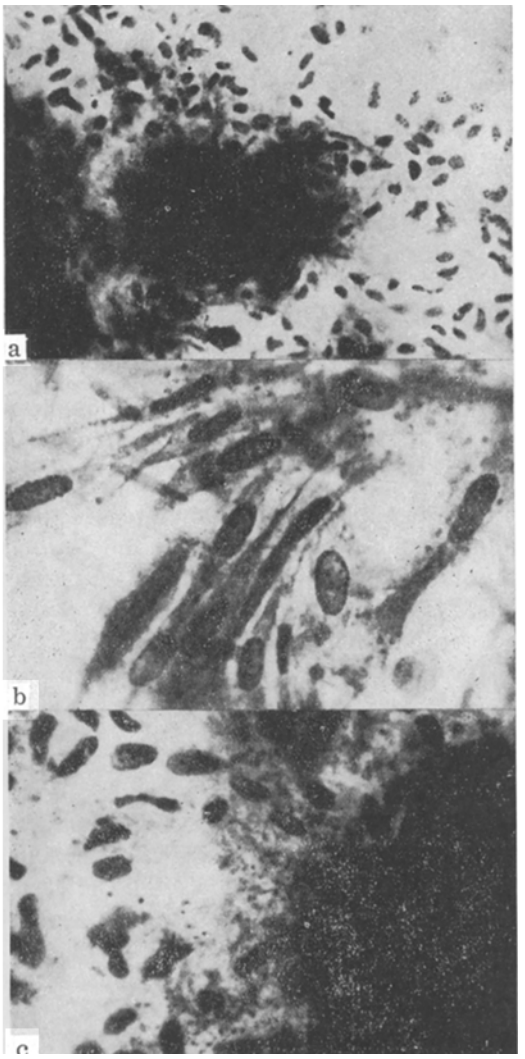

Fig. 2. Contents of diffusion chambers with marrow transplated from irradiated mice. a) 10 days after irradiation: focus of osteogenesis, objective 20; b) 10 days after irradiation: long phosphatase-positive cells of osteogenic focus, objective 40; c) 15 days after irradiation: osteogenic focus, objective 40.

space inside the chambers was filled with fatty tissue. Osteogenesis was observed in 4 of the 14 chambers (Figs. 1b, 2a). The foci of osteogenesis were small and consisted of long cells and a phosphatase-positive ground substance (Fig. 2b). The foci of bone gave a clearly positive reaction for alkaline phosphatase on account of the ground substance and the cells. Other phosphatase-positive foci not possessing a bony structure also were present in the chambers. They consisted of fibroblast-like elements and an amorphous ground substance. Osteogenesis was observed in 4 of the 14 chambers filled with marrow from untreated donors, and in 7 of the 13 chambers filled with marrow from treated donors.

Numerous osteogenic foci and areas of well-developed bone tissue were found in the chambers with marrow taken from donors 15 days after irradiation (Figs. 1 c, 2 c). Development of myeloid tissue was not observed in any of the chambers. If fragments of bone were introduced into the chamber during expiantation,

by the 15th day they were completely degenerated. It is interesting to note that none of the osteogenic foci were adjacent to this necrotic bone. The number of chambers with bone was 8 of 9 in the first case and 6 of 10 in the second.

The results demonstrate that on the 5th day after irradiation in a dose of 750 R the bone marrow of both treated and untreated mice had no osteogenic potential. On the 10th day after irradiation the osteogenic power of the marrow started to recover, and on the 15th day it was nearly normal. A firm correlation was observed between the osteogenic potential of the marrow and the number of cells contained in the femur.

The number of cells from the treated and untreated mice 5 days after irradiation was one-hundredth of normal. This number of cells was presumably too few for the formation of osteogenic foci in the chamber and to establish a solid layer of fibroblast-like cells in it. On the 10th day after irradiation the number of marrow cells in the femur was increased. The number of cells in the irradiated, treated mice was now 2.4 times greater then in the irradiated mice not receiving the suspension of marrow cells (8×10^5 and 1.9×10^6 cells). Correspondingly, the number of chambers with osteogenic foci in the first case was almost twice that in the second. Finally, on the 15th day after irradiation, the number of marrow cells in the femur of the irradiated animals was the same (2.4×10^6 and 1.6×10^7), according to the published data [2], as that which can maintain osteogenesis in chambers of the size which were used in the present experiments. The number of chambers with foci of bone in explants taken from both was about the same. An important factor in connection with the formation of osteogenic foci in the marrow explants is the density of the marrow cells [2]. Disappearance of the osteogenic potential of the irradiated marrow can thus be explained in two ways. 1) Irradiation damages certain osteogenic precursor cells. By the 10th day they regenerate from residual cells of this line up to the number required for osteogenesis to take place in diffusion chambers. 2) After irradiation, restoration of the population of osteogenic precursor cells takes place as a result of differentiation of hematopoietic stem cells. The present investigation indicates experimental ways of solving this particular problem.

LITERATURE CITED

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