OSTEOGENIC POTENTIAL OF THE BONE MARROW OF IRRADIATED MICE REVEALED BY HETEROTOPIC TRANSPLANTATION

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After syngeneic transplantation of bone marrow taken from mice irradiated in doses of 825-1000 rad, bone marrow tissue is formed beneath the renal capsule of unirradiated mice. If the animals are irradiated in doses of 3000 rad or over, scar tissue with remnants of dead bone is found at the site of the graft. After transplantation of bone marrow from donors irradiated in doses of 825 or 870 rad and then either untreated or treated with syngeneic bone marrow or spleen cells, the osteogenic activity of the bone marrow is considerably impaired; from the 4th to the 21st day after irradiation the bone marrow is virtually without osteogenic properties. They are restored by the 26th-30th day.

A previous investigation [1] showed that heterotopic semisyngeneic grafts of mouse bone marrow transplanted beneath the renal capsule contain cells of two lines: hematopoietic, belonging entirely to the recipient after the 3rd month, and osteogenic, belonging to the donor and capable of maintaining itself for at least 14 months. A radiochimera model is best used to continue the analysis of the relationships between these two categories of cells. In this connection information regarding the osteogenic activity of bone marrow grafts from irradiated animals and, in particular, from animals grafted with hematopoietic cells after irradiation, is essential. The present communication examines these questions.

EXPERIMENTAL METHOD

Adult C3H and CBA mice were used for the experiments. Syngeneic bone marrow was grafted from one femur beneath the renal capsule of unirradiated recipients. The bone marrow was expressed from a syringe into a Petri dish with medium No. 199 and grafted beneath the capsule of the left kidney as pieces of tissue. The grafts were fixed in alcohol–formol on the 30th day. The grafts were studied under a binocular loupe; some of them were decalcified and embedded in paraffin wax, after which sections were cut from them and stained by the PAS method and counterstained with hematoxylin. The donors consisted of unirradiated mice, mice irradiated 2 h before removal of the bone marrow in doses of 825-1000, 3000, 6000, and 10,000 rad on a cobalt EKU-50 apparatus (dose rate 275 rad/min), and mice irradiated 4-46 days before removal of the bone marrow in a dose of 870 rad on a cobalt apparatus or in a dose of 825 rad on a type RUP-3 x-ray apparatus at a dose rate of 30 rad/min. After irradiation, some of these mice received an intravenous injection of a suspension of syngeneic bone marrow ($1 \cdot 10^7$-$2 \cdot 10^7$) or spleen ($5 \cdot 10^7$) cells. Until the time of sacrifice, the water which the irradiated mice drank was treated with antibiotics to give concentrations of 100 mg% neomycin and 10 mg% polymyxin. Altogether 158 grafts were used.

EXPERIMENTAL RESULTS

Transplantation from Unirradiated Donors. All 18 grafts 30 days after transplantation had formed a bone-marrow organ. A continuous and well-developed layer of bone was present beneath the thin connective-tissue capsule of the kidney. Small nodules of bone tissue of various shapes were located in the sub-

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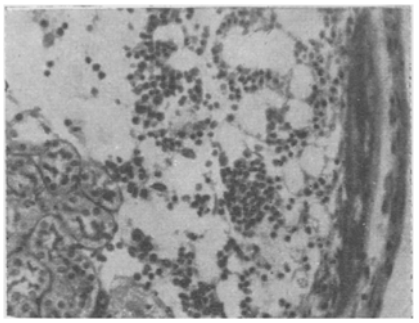

Fig. 1. Heterotopic bone marrow graft from donors irradiated 2 h before the operation in a dose of 825 rad. Bone with bone marrow, 30 days, 20×.

stance of the graft. The bone was mature, with clearly defined living osteocytes and a continuous osteoblastic layer. The space between the kidney and bone tissue was filled by confluent foci of hematopoietic cells.

Transplantation from Donors Irradiated 2 h before Removal of Bone Marrow in Doses of 825-10,000 rad. Bone with bone marrow was present (Fig. 1) in grafts obtained from donors irradiated in doses of 825 and 1000 rad. Three of the five bone marrow grafts from donors irradiated in a dose of 3000 rad consisted of a small quantity of scar tissue. In two other grafts flakes of bone consisting of dead bone tissue were found; the cavities in the bone were empty or filled with fragments of disintegrated nuclei; there was no osteoblastic layer. No bone marrow was present in the grafts. In the place of the grafts taken from donors irradiated in doses of 6000 and 10,000 rad, there were only strips of scar tissue easily separable from the kidney.

Transplantation from Donors Irradiated 4-10 Days before Removal of Bone Marrow in Doses of 825 and 870 rad (Table 1). Scar tissue was present at the site of the graft. In the center of some scars there were small fragments of dead bone tissue, containing neither osteocytes nor an osteoblastic layer. No hematopoietic tissue was present.

Transplantation from Donors Irradiated 4-46 Days before Removal of Marrow in Doses of 825 and 870 rad, and Treated by Injection of Hematopoietic Cells (Table 1). Grafts of bone marrow taken 4-21 days after irradiation contained scar tissue, and in one-third of the cases they also contained fragments of dead bone (Fig. 2). Living, well-differentiated bone was found in all cases of grafting bone marrow 26 days after irradiation. In 8 of these grafts there were small foci of hematopoietic cells.

Grafts of bone marrow taken 30-46 days after irradiation contained living and well-developed bone tissue as well as bone marrow. They did not differ visibly from grafts of normal bone marrow.

TABLE 1. Results of Transplantation of Bone Marrow Taken at Different Times after Irradiation of Donors and of Injection of Hematopoietic Cells into Recipients

Dose of irradiation (in rad)	No. of hematopoietic cells used for treatment	Time between irradiation of donor and transplantation (in days)	Results		
			complete resorption of graft	bone tissue in graft	bone with marrow in graft
825	—	4	1/3	2*/3	—
		7	4/4	—	—
870	—	5	5/5	—	—
		10	5/5	—	—
825	$2 \cdot 10^7$ bone marrow cells or $5 \cdot 10^7$ spleen cells	4	2/3	1*/3	1 †/6
		7	3/6	2*/6	
		12	8/9	1*/9	—
		15—16	11/16	5*/16	—
		20—21	4/10	6*/10	
		26	—	4/7	3 †/7
		30—33	—	—	9/9
870	10^7 bone marrow cells	5	5/5	—	—
		10	7/7	—	—
		12	8/8	—	—
		17	6/6	—	—
		26	—	—	5/5
		46	—	—	6/6

* Scar tissue containing fragments of dead bone.

† Bone tissue with a small quantity of bone marrow.

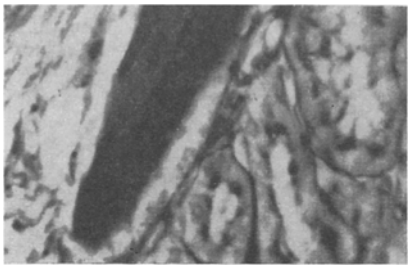

Fig. 2. Heterotopic bone marrow graft from donors irradiated 7 days before the operation in a dose of 825 rad and treated with syngeneic bone marrow. Fragment of dead bone, 30 days, 20×.

These results show that irradiation in vivo in a dose of 1000 rad does not affect the fate of heterotopic bone marrow grafts. After irradiation in a dose of 3000 rad, by the 30th day at the site of the grafts only scar connective tissue with remnants of dead bone can be found. The shape and arrangement of the trabeculae of the bone show that it is formed after transplantation and is not the residual trabeculae of spongy bone transplanted from the donor. This is supported by the absence of dead bone in grafts from donors irradiated in higher doses (6000 and 10,000 rad): only scar tissue was found at the site of such grafts. Fragments of dead bone discovered 30 days after grafting evidently arose from osteogenic cells of the irradiated donor, but they were incapable of maintaining themselves for long. Such bone tissue thus rapidly became spontaneously exhausted.

Irradiation in a dose of 3000 rad thus eliminated the osteogenic cells capable of prolonged self-maintainance which were the precursors of the bone marrow. However, even after such irradiation, cells capable of differentiation into bone cells evidently still remained. With a dose of 6000 rad or more, these cells also were destroyed. Investigations [2, 3] have shown that most self-maintaining (stem) hematopoietic cells die during irradiation in a dose of 1000 rad. The normal osteogenic potential of such bone marrow is therefore evidence that the source of osteogenesis in the bone marrow grafts was a special line of precursor cells and not hematopoietic stem cells taken from the donors. Not immediately after irradiation, but after a lapse of 4-10 days, more severe damage to osteogenic activity was found: after irradiation in a dose of 825 rad, the osteogenic activity of this bone marrow was sharply reduced. This means that self-maintaining osteogenic cells remaining in the bone marrow after irradiation at this level either die during the first 4 days or differentiate in another direction. The bone marrow of donors irradiated and treated with cells of syngeneic bone marrow or spleen between 4 and 21 days after irradiation was virtually without osteogenic properties, but it regained them by the 26th day. The results provide experimental material for analysis of the role of transplanted hematopoietic cells in the restoration of the osteogenic potential of the bone marrow of radiochimeras.

They show, in addition, that, despite its relative radioresistance, the osteogenic tissue of the bone marrow is damaged during the development of radiation sickness.

LITERATURE CITED

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