RESTORATION OF OSTEOGENIC PROPERTIES OF BONE MARROW AFTER IRRADIATION IN HETEROTOPIC TRANSPLANTATION EXPERIMENTS

A. I. Kuralesova

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After subcapsular transplantation of tibial bone marrow, locally irradiated in a dose of 2000 R, into the kidney of mice of the same line 0-4 months after irradiation, resporption always took place. Meanwhile, bone marrow from the unirradiated limb of the same donors always possessed osteogenic potential. The stroma of the irradiated limb was defective and did not regenerate by repopulation of the stromal cells from unirradiated areas. Experiments in which mice were irradiated repeatedly and the same limb was shielded gave similar conclusions. The results of heterotopic transplantation show that stromal cells, unlike hematopoietic cells, cannot repopulate.

KEY WORDS: bone marrow; irradiation; osteogenic potential; heterotopic transplantation.

Heterotopic bone-marrow transplantation experiments have shown that bone-marrow precursor cells with osteogenic potential are histogenetically independent of the hematopoietic and lymphoid stem cells and are capable of self-support for a long time [1, 3, 4].

The object of this investigation was to study whether repopulation of irradiated areas of bone marrow with predetermined osteogeneic precursor cells can take place.

EXPERIMENTAL METHOD

F_1(CBA ×A) and F_1(CBA ×C_{57}BL) mice weighing 18-20 g were used.

Whole-body irradiation of the animals was carried out with the ÉKU-5 cobalt apparatus (dose rate 30 rad/min) and local irradiation with the RUM-3 x-ray apparatus (dose rate 100 rad/min). The marrow donors were killed at different times after irradiation and the marrow was blown out of their femur or tibia in one piece. This bone marrow was transplanted beneath the renal capsule into recipients by the method described previously [5]. Each graft consisted of the contents of one femur or one tibia.

The results of transplantation in all the experiments were noted 1 month after the operation. For this purpose the grafts were studied under a binocular loupe and then fixed in alcohol–formol and decalcified. After embedding in paraffin wax, a series of histological sections were cut and stained with hematoxylin–eosin. The following series of experiments were carried out. I) Transplantation of bone marrow immediately after whole-body irradiation of the donors, at time 0. In some experiments tetracycline was given to the recipients in a dose of 1 mg/20 g body weight 24 h before fixation of the grafts. The grafts were removed next day and examined under the MUF-1 luminescence microscope. II) Transplantation of the tibial marrow from the locally irradiated hind limb at various times after irradiation. Bone marrow from the right hind limb was used for control transplantation. III) Transplantation of bone marrow from donors irradiated with one hind limb shielded. Times of transplantation: 0-32 days after irradiation. IV)

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.)
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TABLE 1. Heterotopic Transplantation of Irradiated Mouse Bone Marrow

Mice	Dose of irradiation (in rad)	Time between irradiation and transplantation	Regeneration of grafts**
F_1 (CBA× ×C_{57}BL)*	700	0- Time‡	6/6
	800	»	6/6
	900	»	5/5
	1000	»	2/6
	1500	»	0/6
	2000	»	0/6
	3000	»	0/5
F_1 (CBA × ×A)†	2000	0—2 h	0/4
		11 days	0/3
		16 days	0/5
		21—22 days	0/6
		41—44 »	0/5
		4 months	0/2

* Subcapsular transplantation of marrow from one femur into kidney.

† Subcapsular transplantation of marrow from one tibia into kidney.

‡ Bone marrow transplanted immediately after irradiation.

** Results of transplantation given as ratio between number of regenerating grafts and total number of grafts at that particular time.

TABLE 2. Heterotopic Transplantation of Bone Marrow from F_1(CBA × C_{57}BL) Mice Receiving Whole-Body Irradiation with Hind Limb Shielded

Dose of irradiation (in rad)	Time between irradiation and transplantation (in days)	Results of transplantation‡
950	0	0/2
	2	0/3
	10	0/3
	21	0/3
	27	2/3
	32	3/3
850 × 2†	7	0/3
	11	0/3
	19	0/2
	27	2/2
	40	3/3

* Each graft included bone marrow from one tibia.

† Interval between two irradiations in dose of 850 rad is 22 days. Bone marrow transplanted after second irradiation.

‡ Results of transplantation given as ratio between number of regenerating grafts and total number of grafts at that particular time.

Transplantation of bone narrow from donors irradiated twice, at an interval of 22 days, with the same hind limb shielded. Bone marrow from the shielded limb was used for the control transplantation. Times of transplantation: 7-40 days after the second irradiation. The doses of irradiation are shown in Tables 1 and 2.

EXPERIMENTAL RESULTS

Bone marrow taken from the femur and tibia of mice immediately after whole-body irradiation in doses of 700, 800, and 900 rad was indistinguishable visually from the marrow of unirradiated animals, but after irradiation in doses of 1000, 1500, 2000, and 3000 rad it was paler than normal, less compact, and more liquid.

One month after transplantation of bone marrow irradiated in doses of 700 and 800 rad (Table 1) under the renal capsule of the recipients, the grafts were strongly luminescent in the ultraviolet microscope. The grafts formed a marrow organ, including a bone shell and active bone marrow. The grafts on mice irradiated in a dose of 900 rad were significantly smaller, but their luminescence in the luminescence microscope remained bright and clear.

Such grafts were present in two of the six recipients receiving bone marrow from donors irradiated in a dose of 1000 rad. On the surface of the kidneys in two cases after transplantation of marrow irradiated in a dose of 1500 rad, small fragments of bone, not shining under the luminescence microscope, could be seen. In all cases in which bone marrow irradiated in higher doses was transplanted, clear resorption of the grafts took place.

Bone marrow from the tibia, irradiated locally in a dose of 2000 rad and transplanted 0 days–4 months after irradiation, always underwent resorption (Table 1). Marrow from the unirradiated tibia of all these donors possessed osteogenic potential, i.e., when grafted beneath the kidney capsule of the recipient, bone with medullary elements was formed.

In the early periods after irradiation (0-21 days), marrow removed from the irradiated limb of animals exposed to a dose of 950 rad with one hind limb shielded lost its osteogenic properties. They were restored in the donors on the 27th day after irradiation, i.e., at the same time as in radiation chimeras [2, 4]. Heterotopic transplantation of marrow irradiated twice in a dose of 850 rad showed that it did not possess osteogenic properties on the 7th-19th day after the second irradiation, but that they were restored 27 days later (Table 2).

In the control series of experiments marrow was transplanted from the shielded limb of these mice. From the 11th to the 40th day after the second irradiation, the marrow was found to possess osteogenic potential.

After syngeneic subcapsular transplantation of bone marrow into the kidney, bone tissue was formed at the site of the graft and was repopulated by the recipient's marrow cells. Osteogenic tissue forming the stroma of these grafts preserved its donor origin, whereas the hematopoietic tissue by the third month was replaced by the recipient's hematopoietic cells. The presence of a potent stroma in these experiments guaranteed the success of transplantation. On the other hand, grafts of marrow, consisting of stroma injured after local irradiation in a dose of 900 rad, together with normal hematopoietic tissue are reported to have undergone resorption [6].

The results of the present experiments show that the lost osteogenic potential of the bone marrow in the irradiated animals can be restored within a month after irradiation in doses of 850-950 rad. The dose causing loss of osteogenic potential of the marrow in mice was shown to be 2000 rad. As transplantation of marrow from a limb locally irradiated in a dose of 2000 rad showed, restoration of these properties did not take place even within 4 months, although the marrow was outwardly indistinguishable in appearance at that time from normal.

The results show that, first, after irradiation of the stroma in a dose of 2000 rad, it is repopulated by hematopoietic cells although the stroma itself remains defective. Second, they show that repopulation of irradiated areas with osteogenic stromal cells from unirradiated areas does not take place (at least in the course of 4 months).

After repeated irradiation of mice with the same limb shielded, repopulation of areas where intensified regeneration was taking place, i.e., the irradiated limb, with stromal cells from the shielded limb could not be obtained. The times of restoration of the osteogenic potential of the marrow from the irradiated limb remained the same as after a single irradiation, i.e., the presence of a "reserve" of stroma in the unirradiated area did not affect them.

The results show that stromal cells in mice cannot repopulate even during repair processes after irradiation.

These results do not contradict those obtained by Crosby et al. [7, 8], who described secondary aplasia of the marrow in rats three months after local irradiation in a dose of 4000 rad. It is evidently at this time that radiation damage to the stroma is manifested and, since repopulation with stromal cells from unirradiated areas does not take place, the stroma does not regenerate.

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