HETEROTOPIC GRAFTS ON BONE MARROW OF TOLERANT ANIMALS

A. I. Kuralesova

UDC 612.6.02 :612.419

Bone marrow was transplanted from CBA mice beneath the capsule of the kidney into line A mice tolerant to CBA. Hematopoietic tissue was taken from the grafts on the 52nd and 83rd days and implanted beneath the capsule of the kidney of lines A and CBA mice. By the 30th day after the operation, the grafts in the A mice contained fragments of dead bone, while those in the CBA mice contained newly formed bone, repopulated with hematopoietic elements. After heterotopic transplantation of bone marrow, induction of bone tissue from the recipient's cells thus does not take place.

* * *

In a previous paper [1] the writer suggested an experimental model which could be used to determine the origin of cells forming the osteogenic stroma of bone marrow after heterotopic grafting. The line of stromal osteogenic cells was maintained for at least 14 months during semisyngenic transplantation of bone marrow in accordance with the A $\rightarrow F_1 \rightarrow$ A scheme, i.e., it retained its donor's nature whereas the hematopoietic cells were replaced by recipient's cells.

To use this model in subsequent research aimed at elucidating possible connections between lines of stromal and hematopoietic cells, it was necessary to determine whether bone tissue is formed after heterotopic grafting not only by differentiation of osteogenic precursor cells present in the graft [1], but also as a result of induction of bone from local connective-tissue cells of the recipient, in contact with the graft. This problem has arisen as a result of observations indicating induction of bone tissue by various methods, including by bone tissue [3-5].

EXPERIMENTAL METHOD

Experiments were carried out on mice of lines A and CBA. To obtain tolerant animals, new born line A mice received an injection of $5 \cdot 10^6$ spleen cells of an adult CBA female into a cervical vein. To determine the presence of tolerance, skin from CBA females was grafted on to the mice by the method of Billingham and Medawar [2], as improved in the IÉKO Virology Laboratory.

Bone marrow removed from the femora of CBA mice was grafted beneath the capsule of the kidney of these mice on the 30th-45th day after birth. A bone marrow organ was found beneath the kidney capsule 52-83 days later. Marrow was extracted from the grafts, divided into several equal parts, and regrafted beneath the kidney capsule of mice of lines A and CBA. On the 30th day the grafts were fixed in alcohol-formol and decalcified. Tests were carried out on histological sections for polysaccharides (staining with hematoxylin). Altogether 11 regrafts from CBA mice (6 aged 52 days, 5 aged 83 days) and 11 regrafts from line A mice (7 aged 52 days, 4 aged 83 days) were investigated (Table 1).

The problem considered in this investigation was thus examined in experiments in which allogenic recipients, tolerant to the donor's line, were used as intermediate host, and mice syngenic to the first recipients, but not tolerant to the line of the original donor, were used as secondary recipient. [Scheme of experiment: CBA $\rightarrow$ A (tolerant to CBA) $\rightarrow$ A.]

Laboratory of Immunomorphology, 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.) Translated from Byulletin Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 7, pp. 97-100, July, 1970. Original article submitted October 9, 1969.

TABLE 1. Results of Regrafting of Bone Marrow from Heterotopic Grafts of Tolerant Animals

Line of primary donor	Line of primary recipient	Time during which graft remained in tolerant animal (in days)	Line of secondary recipient	Time during which graft remained in secondary recipient (in days)	Results of regrafting	
					resorption	bone + bone marrow
CBA	A	52	A	30	7/7	–
			CBA	30	–	6/6
		83	A	30	4/4	–
			CBA	30	–	5/5

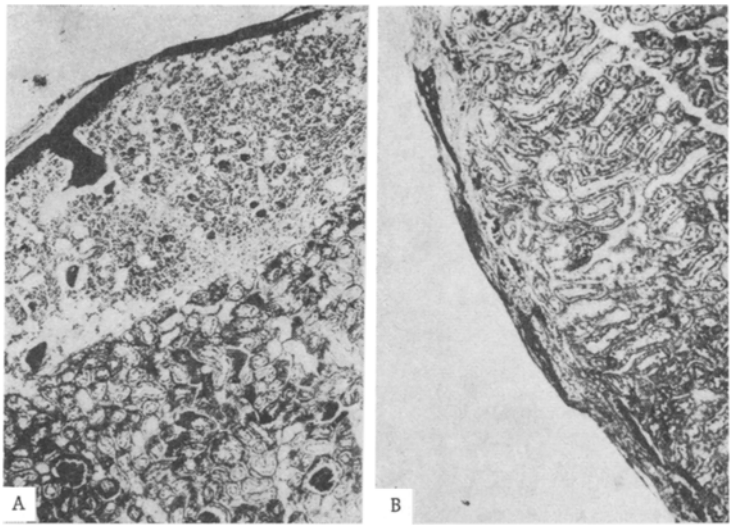

Fig. 1. Regrafting of hematopoietic tissue of 52-day heterotopic bone marrow grafts of tolerant animals. Time of fixation 30 days; PAS reaction and hematoxylin; 208×. A) Regrafting of bone marrow on donor's line; newly formed bone with bone marrow; B) regrafting of bone marrow on recipient's line; fragments of dead bone.

EXPERIMENTAL RESULTS

On the 30th day after grafting bone marrow from 52-day grafts on the parent CBA line the same morphological picture was observed as in syngenic grafts [1], i.e., in every case bone and bone marrow were present (Fig. 1A). The bone was living and differentiated, reacting positively for polysaccharides. As a rule the bone trabeculae were superficial in position. Small bone lamellae also were present in the depths of the graft. Bone cavities enclosed in ground substance were occupied by living osteocytes. The osteoblastic layer of bone was clearly defined. The space between the bone trabeculae and the kidney was filled with active bone marrow, including elements of the myeloid and erythroid series and megakaryocytes.

When bone marrow from 83-day grafts was transplanted on to the parent line, no morphological differences from grafts at the preceding time could be detected 30 days after operation.

After regrafting of bone marrow from 52- and 83-day grafts on to line A mice, these regrafts were similar in histological structure to allogenic bone marrow grafts [1]. All grafts were flat and small. Fragments of dead, resorbed bone were present in the substance of the dense connective tissue.

The bone cavities were empty and the osteoblastic layer absent. No bone marrow was found in such grafts (Fig. 1B). All grafts of allogenic CBA bone marrow implanted beneath the kidney capsule of line A mice tolerant to CBA thus survived in the recipient. Conditions created by heterotopic grafting were fav-

orable for preservation of the line of stromal cells giving rise to bone. This is clear from the fact that when bone marrow was regrafted by the scheme CBA → A (tolerant to CBA) → CBA, viable bone was formed in the grafts. These results confirm those obtained previously [1].

If bone formed in heterotopic grafts of bone marrow present in the primary recipient could have induced bone tissue from surrounding connective tissue elements, after regrafting of these elements in the line A animals (the recipient's line), they would have survived. It must be remembered that addition of allogenic bone marrow to syngenic as an impurity does not prevent the characteristic differentiation of such grafts [1]. In the present experiments all regrafts on line A were completely resorbed. This fact indicates that induction of bone does not take place in heterotopic bone marrow grafts.

The degree of chimerism of the tolerant animals and the degree of repopulation of the graft by the recipient's hematopoietic cells remained unexplained. For this reason, the experiments do not answer the question whether hematopoietic cells can replenish the pool of precursor cells for osteogenic tissue. This question is to be studied in future investigations.

LITERATURE CITED

1. A. Ya. Fridenshtein, K. V. Petrakova, A. I. Kuralesova, et al., Transplantation, 6, 245 (1968).
2. R. E. Billingham and P. B. Medawar, J. Exp. Biol., 28, 385 (1951).
3. P. P. H. De Bruyn and W. T. Kabisch, Am. J. Anat., 96, 375 (1955).
4. M. R. Urist and F. C. McLean, J. Bone Jt. Surg., 34A, 443 (1952).
5. M. R. Urist, Science, 150, 898 (1965).