BONE MARROW ORGAN FORMATION AFTER TRANSPLANTATION OF CELL SUSPENSIONS INTO SPONGES

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It was established about a century ago that heterotopic hematopoietic organs are formed after transplantation of fragments of bone marrow [7]. As a result of transplantation, the hematopoietic microenvironment is transferred through survival of medullary stromal mechanocytes, which constitute a small fraction among the remaining bone marrow cells [5]. In fact, it is the stromal mechanocytes which preserve their donor origin in semisyngeneic heterotopic transplants [3], and during transplantations of chimeric bone marrow, in which stromal mechanocytes and hematopoietic cells are not identical with respect to H-2 allotypes, transfer is possible only to a line of recipients suitable to allow survival of the stromal mechanocytes [4-6].

To carry the analysis of mechanisms of transfer of the hematopoietic microenvironment a stage further it is important to transplant, not fragments of bone marrow, but cell suspensions and to vary the composition of the transplanted cells and their mutual arrangement. The writers showed previously that this problem can be solved, in principle, by transplanting bone marrow cells deposited on the surface of millipore filters [2].

In the present investigation transfer of the bone-marrow microenvironment was studied when bone marrow cells were transplanted into sponges.

EXPERIMENTAL METHOD

Adult CBA and (CBA × C57BL)F_1 mice were used. Bone marrow was flushed out by means of a syringe into medium No. 199 from the femora after amputation of the epiphyses. Cell suspensions were prepared by mincing the bone marrow tissue on a magnetic mixer for 30 min at room temperature in medium No. 199 or in 0.25% trypsin solution. The resulting suspensions were filtered through four layers of nylon, to give unicellular suspensions. These were treated with 30% embryonic calf serum and the cells were sedimented by centrifugation and resuspended in medium of the same composition to a concentration of about 5×10^7 cells/ml. The necessary volume of suspension was introduced into 0.1-ml conical plastic tubes, the cells were sedimented by centrifugation and resuspended in 0.2 ml medium No. 199, and this suspension was used to soak sponges.

Two types of sponges were used: gelatin (Gelfoamsponge) and collagen (made from a 1.7% solution of collagen acetate from calf skin by freeze drying). Collagen sponges were subjected to mild tanning in formalin vapor (3 h) or strong tanning in 1% glutaraldehyde solution (24 h), after which they were washed in water (24 h) and dried. The dry sponges were sterilized with UV light. Pieces of dried sponges (weighing 0.1-0.8 mg) were moistened before use with medium No. 199, squeezed between filter paper, and then placed in tubes containing the cell suspension. Under these circumstances the sponge straightened out and completely absorbed the cell suspension. Sponges with bone marrow cells were grafted beneath the kidney capsule of syngeneic recipients. In the control, empty sponges or sponges with bone marrow cells previously irradiated in a dose of 6000 rads were transplanted. The grafts were fixed on the 9th-80th day with alcohol-formol, decalcified, and used to make histological preparations. Series of sections were stained with hematoxylin and eosin and by the PAS method.

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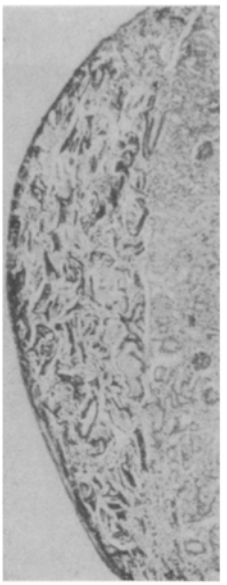

Fig. 1

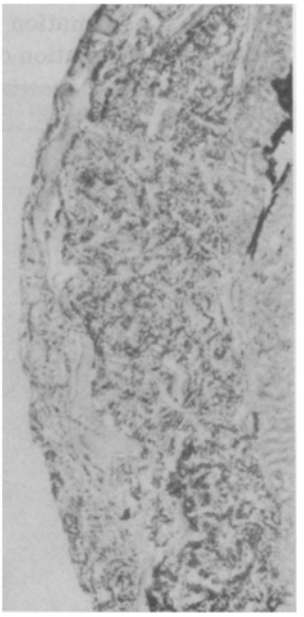

Fig. 2

Fig. 1. Graft of 2×10^7 trypsinized bone marrow cells in gelatin sponge weighing 0.1 mg after 16 days. Objective 4×.

Fig. 2. Graft of 10^6 trypsinized bone marrow cells in gelatin sponge weighing 0.6 mg, after 45 days. Objective 4×.

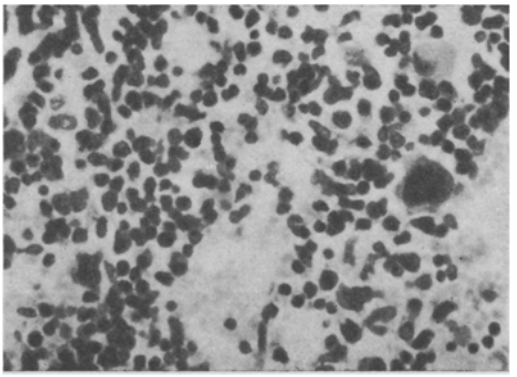

Fig. 3. The same preparation, objective 24×.

EXPERIMENTAL RESULTS

The number of cells released by mincing bone marrow in medium No. 199 was about 8×10^6, and in trypsin solution about 1×10^7, cells from the medullary cavity of one femur. The composition of the suspension (from the cell formula determined in films) likewise was the same. Control sponges (empty or filled with irradiated cells) gave the same results after implantation. Gelatin and strongly tanned collagen sponges kept well for up to 2 months, although they shrank after the 30th day. Connective tissue developed gradually inside the sponges, as described previously [1]. The weakly tanned collagen sponges were absorbed as early as by the 2nd week.

Transplantation of 6×10^5 to 24×10^6 cells of trypsinized and 15×10^6 cells of nontrypsinized bone marrow into gelatin sponges led to the formation of bone tissue inside the sponges. By the 16th day the sponges were clear of hematopoietic cells (Fig. 1). Reticular tissue with wide capillaries developed in the pores of the sponges, and bony trabeculae were formed on the side facing the kidney. The bones were much larger in grafts of trypsinized cells. By the end of the first month the lower (facing the kidney) surface of the grafts

TABLE 1. Formation of Bone Marrow Organs after Transplantation of Unicellular Bone Marrow Suspensions into Gelatin Sponges

Cells transplanted	Number of cells transplanted	Time of fixation, days	Ratio of number of grafts with bone to total number of grafts	Ratio of number of grafts with bone and bone marrow to total number of grafts
Trypsinized	$6 \cdot 10^5$	45	6/6	6/6
	10^6	45	5/5	5/5
	$3 \cdot 10^6$	45	5/5	5/5
	$15 \cdot 10^6$	42	3/3	3/3
	$18 \cdot 10^6$	50	5/5	5/5
	$24 \cdot 10^6$	27—50	3/3	3/3
Untrypsinized	$15 \cdot 10^6$	47—80	6/27	0/27
Sponges without cells or with $10 \cdot 10^6$ to $20 \cdot 10^6$ irradiated cells	—	47—60	0/26	0/26

with trypsinized cells was covered with a shell of bone, and the central part of the sponge was filled with spongy bone, blood vessels of sinusoidal type, and foci of hematopoiesis; the tissue of the sponge showed a considerable degree of absorption. By the 42nd-50th day, in place of the sponge and reproducing its shape and size, a bone marrow organ containing medullary cavities with actively hematopoietic bone marrow was formed, covered above with bone (Figs. 2 and 3). The time taken for complete formation of such an organ and its size depended (given sponges of constant dimensions) on the number of transplanted cells. In grafts of untrypsinized marrow cells typical bone marrow organs as a rule were not formed (Table 1). Only isolated foci of bone tissue were observed in the sponges even after 80 days, no medullary cavity was formed, and n ch of the sponge was filled with reticular and scar tissue.

After transplantation of trypsinized bone marrow cells into collagen sponges the results depended directly on the degree of tanning of the sponges. When weakly tanned sponges were used transplantation even of $1{,}5 \times 10^7$ to $2{,}0 \times 10^7$ cells was ineffective: the grafts were absorbed together with the sponge. Only in isolated recipients were small degenerating bone fragments found on the surface of the kidney at the 45th day. In strongly tanned sponges, however, the grafts developed in the same way as in gelatin sponges and were highly effective as regards the formation of bone marrow organs.

On the whole, after transplantation of suspensions of bone marrow cells into sponges bone tissue developed highly effectively and a bone marrow microenvironment was formed. As the investigation showed, morphogenesis of bone and other structures of the bone marrow stroma requires the presence not only of stromal precursors in the grafts, but also of a definitely organized framework in which the cells can grow; no bone marrow organ was formed in rapidly absorbed sponges. The framework also determines the shape and limits the size of the future organ, as will be discussed in a special communication. Trypsinized bone marrow was much more effective as regards bone formation and transfer of the hematopoietic microenvironment. The cells responsible for both these processes evidently have more stable contacts than hematopoietic cells. However, it is also possible that the bone marrow stroma includes subpopulations of mechanocytes, differing in their morphogenetic potential and in the strength of their intercellular contacts.

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