MAINTENANCE OF HEMATOPOIESIS IN BONE MARROW TRANSPLANTED INTO PRELIMINARILY CULTIVATED BONE STROMA

T. O. Prusevich and E. A. Luriya

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Maintenance of differentiation of bone marrow in organ cultures in observed for 5 days. If bone marrow fragments are cultivated together with preliminarily grown osteogenic tissue, intensive myeloid hematopoiesis is maintained until the 16th day. The bone stroma thus exerts its influence on the hematopoietic tissue, helping to maintain differentiation and proliferation of hematopoietic cells.

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In submerged cultures of hematopoietic tissue (monolayer cultures or cultures grown in a plasma clot) hematopoietic cells disappear during the first few days of cultivation, and proliferation of stromal cells is observed [3, 4, 7, 8]. Methods of organ cultivation ensured longer preservation of hematopoietic cells. If fragments of bone marrow from adult mice are explanted on millipore filters, intensive hematopoiesis is observed for the first 5 days [2]. In cultures of a mouse bone marrow suspension on agar, the formation of foci of myeloid cells was observed if serum from leukemic AKR mice was added to the medium, and the myeloid cells survived for 5 days [6]. Longer hematopoiesis (10-12 days) was obtained in bone marrow cultures on agar if a stroma of preliminarily cultivated kidney cells was used [5]. Intensive myeloid and megakaryocytic hematopoiesis was observed for 24 days in organ cultures of mouse embryonic liver [2]. Methods of organ cultivation in which the explant is introduced on the boundary between two phases have the distinguishing feature that the tissues are well aerated and, under such conditions, the natural interactions between the cells characteristic of the particular tissue concerned are preserved. Cell interactions between hematopoietic and stromal cells play an important role in the maintenance of differentiation of hematopoietic tissue in vivo [9].

These considerations form the basis for the present investigation in which differentiation of hematopoietic cells of the bone marrow was studied during combined cultivation with osteogenic tissue.

EXPERIMENTAL METHOD

The tissue was grown in organ cultures by the method described previously [1] on AUFS millipore filters (pore diameter 0.6-0.9 μ), placed above a liquid nutrient medium consisting of medium No. 199, 20% bovine serum, and 10% chick embryonic extract. To each 100 ml medium, 400 g glucose, 15 mg vitamin C, 20 mg L-glutamine, and 5000 units each of penicillin and streptomycin were added. Fragments of bone marrow from 17-day mouse embryos, freed from surrounding tissues, were explanted. After 10-14 days, fragments of bone marrow taken from the femora of adult noninbred mice were explanted onto the same filters. One-third of the contents of each femur was transplanted.

The medium was changed every 48-72 h. Cultures were fixed in 96° alcohol before transplantation of bone marrow or 6, 7, 8, 9, 11, 14, 16, and 18 days after transplantation. At each time 3-5 cultures were fixed. The bone fragments were carefully removed from the filters, efforts being made not to damage the zone of growth, and the material was cut into series of sections which were stained with alum-hematoxylin. Gomori's reaction for alkaline phosphatase was carried out on filters fixed before transplantation of bone marrow. The remaining filters were stained with hematoxylin, dehydrated and cleared, and then mounted in Canada balsam as total preparation.

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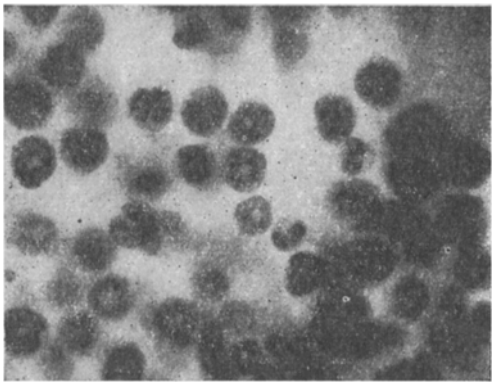

Fig. 1. Myeloid hematopoiesis in an 11-day bone marrow culture explanted on a bone stroma. Total preparation. Hematoxylin, 80×.

EXPERIMENTAL RESULTS

Osteogenesis was observed in the bone fragments on the 10th-14th day in vitro, and the zone of growth on the filter included osteogenic tissue giving a positive reaction for alkaline phosphatase. Morphologically mature bone trabeculae were not observed in the zone of growth at these periods.

On the 6th-8th day of combined cultivation of bone marrow and embryonic mouse bone, areas of completed osteogenesis, consisting of bone trabeculae with deposition of calcium, were found in the zone of growth adjacent to the bone explant. The peripheral parts of the zone of growth did not exhibit any clearly defined morphology of bone tissue but consisted of regularly arranged bands of cells without deposition of ground substance. Hematopoietic cells were concentrated mainly around the explanted piece of bone and were arranged as compact groups above the stromal cells.

The greater part of the explanted piece of bone marrow was dead on the 6th day. In the bone fragment itself osteogenesis was observed at the periphery. Repopulation of the newly formed bone by hematopoietic cells was not observed in any case examined. On the 9th and, in particular, the 11th day of explantation, intensification of osteogenesis and myeloid hematopoiesis was observed in the cultures (Fig. 1). The stratified zone of growth around the bone explant contained numerous branching bone trabeculae in which calcium was being laid down in places. Mature bone structures could be seen in the foci of osteogenesis: a peripheral layer of osteoblasts and immured osteocytes. Stratified foci of myeloid hematopoiesis consisting of several hundreds of cells were in contact with the bone trabeculae. In the superficial layer of the zone of growth, where no morphologically clearly defined osteogenesis was present, large areas of intensive myeloid hematopoiesis also were present, and mitotically dividing cells were seen among the myeloid elements. Foci of myeloid cells also spread at the periphery of the zone of growth, covering a considerable area.

In cultures 14-16 days old the number of hematopoietic cells was rather smaller than at the preceding stage. Myeloid cells at various stages of differentiation formed foci of different sizes around the explant and at the periphery of the superficial layer of the zone of growth. On the 18th day after transplantation of bone marrow, viable myeloid cells were occasionally found in the specimens. Hence, prolonged and intensive hematopoiesis is observed in an organ culture of mouse bone marrow explanted on to preliminarily grown osteogenic tissue, and persists for 16 days. If bone marrow is explanted into an organ culture directly on millipore filters, hematopoiesis is observed for 5 days [2].

The results described indicate that osteogenic tissue exerts an influence on bone marrow explants, helping to maintain differentiation and proliferation of hematopoietic tissue. Intensive hematopoiesis takes place in the surface layers of the zone of growth formed by the osteogenic tissue. Foci of hematopoietic cells frequently make contact with areas of differentiated bone in the zone of growth. Repopulation of the explanted bone fragment by hematopoietic cells never took place in any of the cases studied.

It is not yet clear whether direct cell contact between the hematopoietic elements and osteogenic tissue is necessary or whether they can interact humorally. Nor is it known at which of the successive stages of histogenesis of hematopoietic tissue the bone stroma exerts its action, and what is the precise nature of this action.

LITERATURE CITED

1. E. A. Luriya and I. E. P'yanchenko, Byull. Éksperim. Biol. i Med., No. 7, 81 (1967).
2. E. A. Luriya, R. D. Bakirov, T. A. Eliseyeva, et al., Exp. Cell Res., 54, 111 (1969).
3. E. A. Luriya, O. V. Chakhava, and A. Ya. Fridenshtein, Tsitologiya, No. 1, 115 (1966).
4. N. G. Khlopin, General Biological and Experimental Bases of Histology [in Russian], Leningrad (1946).
5. T. R. Bradley and D. Metcalf, Aust. J. Exp. Biol. Sci., 44, 287 (1966).
6. T. R. Bradley, D. Metcalf, and W. Robinson, Nature, 213, 926 (1967).
7. A. Carrel and M. Burrows, J. Am. Med. Assn., 55, 1379 (1910).
8. P. Farnes and B. Barker, Exp. Cell Res., 29, 278 (1963).
9. C. E. Ford, P. L. T. Ebery, and J. F. Loutit, J. Cell. Comp. Physiol., 50, Suppl. 1, 123 (1957).