BONE TISSUE FORMATION IN HUMAN BONE MARROW ORGAN CULTURES

E. A. Luriya, S. A. Kuznetsov,
E. N. Genkina, and A. Ya. Fridenshtein

UDC 611.018.46.086.83

KEY WORDS: bone marrow; man; organ cultures; osteogenesis

A cultural system in which mouse bone marrow fragments from bone tissue in vitro [2], very similar in its morphological and ultrastructural characteristics to native mouse bone tissue, has recently been described [4]. Similar results were obtained by culturing mouse bone marrow cell suspensions [3]. This paper gives data on bone tissue formation in cultures of human bone marrow fragments and cell suspensions.

EXPERIMENTAL METHOD

The sources of the bone marrow were ribs from patients aged 20-55 years undergoing operations for tuberculosis, empyema, and kyphoscoliosis; processes and bodies of vertebrae from patients aged 6-40 years undergoing operations for scoliosis; ribs from persons dying from causes unconnected with bone marrow pathology, not more than 7 h before the beginning of explantation. Bone marrow isolated from the medullary cavity was explanted in the form of fragments measuring 3-8 mm^3 (the aim was to avoid introducing any bone fragments), or suspensions of disaggregated cells [3]. Explantation was carried out on the surface of HAWP millipore filters, cut into squares with an area of 64 mm^2, by the multiple organ cultures method [1]. During explantation of the cell suspensions, a piece of gelatin sponge 0.5 mm thick and with an area of 25-30 cm^2 was placed on each filter [3]. The culture medium had the following composition: 80% of medium MEM (α), 20% of human serum of blood group IV. The additives (per 100 ml) comprised: L-glutamine (50 mg), glucose (400 mg), vitamin C (15 mg), penicillin and streptomycin (6000 U of each). Cultivation was conducted at 37°C in an atmosphere consisting of a mixture of air with 5-7% CO_2 and saturated with water vapor. The culture medium was replaced twice a week. On the 5th-15th day, 10 mM sodium β-glycerophosphate and 10^{-7} M hydrocortisone were added to the medium. The cultures were fixed with ethanol on the 5th-51st days. Total preparations and serial paraffin sections were stained for alkaline phosphatase by Gomori's method and for insoluble calcium salts by Von Kossa's method and with alum-hematoxylin.

EXPERIMENTAL RESULTS

Cultures of Bone Marrow Fragments. During the first days of culture many hematopoietic cells and macrophages from the explants settled on the filter. By the end of the first week single fibroblasts appeared. They proliferated rapidly and in the course of 2 weeks they became the predominant cell, forming a circular zone of growth, covering the greater part of the filter. By the end of the 2nd week the fibroblastic zone of growth had become stratified. The number of hematopoietic cells was reduced, but in part of the cultures foci of myeloid cells lying above fibroblasts still remained even until the 6th week of culture. By the end of the 3rd week, regions of cellular compaction appeared in the central parts of the zone of growth, where the formation of ground substance and deposition of insoluble calcium salts were beginning. Later (5th-6th weeks) the deposits of mineralized ground substance became extensive and massive. As a rule a layer of round, cubical, or columnar osteoblasts lay next to the filter, into the pores of which processes of the cells penetrated deeply. Above this

Laboratory of Immunomorphology, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Laboratory of Medical Biophysics, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Prozorovskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 593-595, May, 1989. Original article submitted July 8, 1988.

 0007-4888/89/0005-0684$12.50

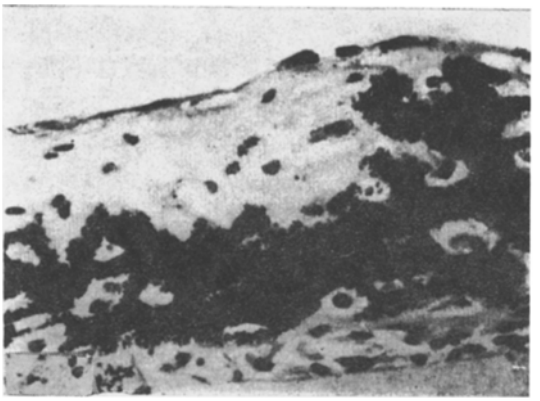

Fig. 1

Fig. 2

Fig. 1. Mineralized bone in organ culture of human bone marrow fragment. 29 Days. Section. Von Kossa's reaction. Objective 24 ×.

Fig. 2. Bone in organ culture of human bone marrow fragment. 29 Days. Section. Gomori's reaction for alkaline phosphatase. Objective 24 ×.

layer, the ground substance of the newly formed bone tissue was up to 100 μ in thickness. It was composed of coarse fibers and contained many chaotically distributed cavities, in which osteocytes were immured. The central parts of the ground substance were abundantly impregnated with insoluble calcium salts (Fig. 1). Above lay an incomplete layer of osteoblasts, and the whole structure was covered by a layer of fusiform fibroblasts.

Toward the periphery the total number of layers, the thickness of the ground substance, and the degree of its mineralization gradually decreased. Calcium salts also were deposited in the thickness of the millipore filter. In zones of intensive osteogenesis, all the cellular layers, the ground substance, and also the filter to a great depth, exhibited high alkaline phosphatase activity (Fig. 2).

The morphological pictures of osteogenesis could vary very considerably. Sometimes the ground substance of the newly formed bone was separated from the filter by fibroblasts. In some cultures a stratified structure developed, consisting of layers of ground substance, alternating with layers of fibroblasts, in the manner of a layer cake; these structures attain a considerable thickness (up to 300 μ). In other cases the ground substance consists of thin (a few millimeters thick) bands, lying in the upper sublayers of the fibroblastic layer. Ground substance may be deposited around fragments of old, lamellar bone, introduced during explantation. The newly formed bone is distinguished by the abundant and haphazard arrangement of the bony cavities, the presence of living osteocytes in them, an osteoblastic layer, a coarse fibrous structure, and more marked metachromasia of the ground substance.

A particular feature of human bone marrow cultures is the abundance of multinuclear giant cells, evidently osteoclasts. More frequently they lay directly on the filter, but if the newly formed bone tissue was removed from the filter, they moved up toward the ground substance. Osteoclasts often were closely apposed to fragments of old lamellar bone, or even filled a lacuna in the ground substance. In vitro, just as in vivo, osteoclasts can evidently take part in resorption of bone tissue. However, no clear correlation could be found between the intensity of osteogenesis in the cultures and the number of osteoclasts.

Parameters of culture have a marked effect on the course of osteogenesis in vitro. The use of medium 199, preliminary heating (up to 56°C) of the human serum, and the use of bovine embryonic serum evidently do not favor osteogenesis. Addition of hydrocortisone and glycerophosphate to the culture medium at the proper time is essential for consolidation of the zone of growth and mineralization of the ground substance of the newly formed bone.

In cultures of mouse bone marrow osteogenesis takes place without the addition of hydrocortisone [2, 4]. Other distinctive features of cultures of human bone marrow fragments include the greater thickness of the newly formed bony lamina, and also the diversity of morphological variants of osteogenesis. They may be caused by insufficient standardization of the explanted tissue, although no definite consistent relationship could be found between the morphology of the cultures and the origin of the bone marrow.

Cultures of Disaggregated Bone Marrow Cell Suspensions. Trypsinized suspensions are explanted in a number of 2.5×10^6-4.5×10^6, and mechanical suspensions in a number of 5×10^6-15×10^6 cells per culture. Just as in the case of cultures of fragments, an extensive stratified fibroblastic zone of growth developed on the surface of the filter, with macrophages, multinuclear giant cells, and fields of myeloid cells on the surface. By the 4th week deposits of insoluble calcium salts began to appear; later (5th-6th weeks) they increased in number, but were slow to compact. In cultures such as these, a fibroblastic zone with remnants of gelatin sponge lay on the filter. In the thickness of this zone, most frequently closer to the surface layers, thin (a few microns thick) layers of ground substance were arranged parallel to the surface of the filter and were impregnated with insoluble calcium salts. Solitary osteoblasts lay alongside.

The suggested system of culture on the whole supports bone tissue formation after explantation of human bone marrow fragments. Osteogenesis in vitro makes it possible to study the effect of various factors on bone tissue formation. Qualitative evaluation of the osteogenic potential of human bone marrow under normal and pathological conditions, and the choice of osteogenetically active preparations for the treatment of particular cases of bone pathology become possible. Interesting opportunities are provided for the in vitro study of osteoclast function.

At the same time, however, osteogenesis in cultures of disaggregated cell suspensions of human bone marrow does not lead to the formation of large and well organized bone structures, as it does in cultures of mouse bone marrow cell suspensions [3] and of human bone marrow fragments, but it ends at the stage of deposition of thin laminae of ground substance. If the suggested system can be improved in this direction, it will then be possible to use bone marrow puncture material for explantation, and this will widen the field of application of the method very considerably. It will also provide an approach to the quantitative analysis of the osteogenic potential of human bone marrow in vitro.

LITERATURE CITED

1. E. A. Luria, Hematopoietic and Lymphoid Tissue in Culture [in Russian], Moscow (1972).
2. E. A. Luria, M. E. Owen, A. Ya. Fridenshtein, et al., Byull. Éksp. Biol. Med., 101, No. 4, 481 (1986).
3. E. A. Luria, S. A. Kuznetsov, E. N. Genkina, and A. Ya. Fridenshtein, Byull. Éksp. Biol. Med., 105, No. 6, 720 (1988).
4. E. A. Luria, M. E. Owen, A. Ya. Fridenshtein (A. J. Friedenstein), et al., Cell Tissue Res., 248, 449 (1987).