OSTEOGENIC PRECURSOR CELLS IN REPARATIVE OSTEOGENESIS

L. N. Mikhailova and A. A. Pal'tsyn

UDC 616.71-003.93-07:616.71-018.1

KEY WORDS: regeneration; osteogenesis; bone marrow tissue; osteogenic precursor cells.

In reparative osteogenesis, just as in repair of other organs and tissues, an important problem is that of the sources of origin of the regeneration blastoma. According to some workers [1], information on sources of regeneration is essential to the investigation of regeneration histogenesis.

During physiological and reparative regeneration of bone tissue the osteoblast population is known to be replenished by proliferation and differentiation of preosteoblasts, which already have well-marked osteogenic potential. However, the earlier precursors of osteogenic cells have not yet been discovered.

With the accumulation of new facts and the further development of our knowledge of osteogenesis, a new theory of osteogenic cells has been put forward, according to which they are precursors of bone marrow stromal cells [9]. Among the stromal cells of bone marrow cells with osteogenic potential have been found, and can be defined as determined osteogenic precursor cells. Cells of a second type have been defined as inducible osteogenic precursor cells. However, neither type has been identified morphologically.

Processes of osteogenesis, whether during regeneration or after transplantation, always begin with invasion of the zone of osteogenesis by blood capillaries and sinusoids, along the course of which lie undifferentiated connective-tissue cells. For example, besides invasion of blood vessels, the development of reticular tissue with dilated vascular sinuses and with the appearance of cells of the hematopoietic series begins in foci of enchondral osteogenesis. This suggested that perivascular cells may be directly related to the processes of histogenesis taking place, including osteogenesis [2, 3].

In a study of biopsy material from regenerating tissue in Ilizarov's transosseous osteosynthesis, it was shown by electron-microscopic autoradiography that cells forming the wall of blood vessels of capillary type, and also cells located around them [6] possessed the highest proliferative activity in regenerating bone.

The aim of the present investigation was to discover osteogenic precursor cells appearing in the medullary cavity after removal of bone marrow. For this purpose we modified a model of medullary curettage. Compared with other models described in the literature, this permits all bone marrow cells to be completely removed, and the tissue fluid to escape from the bone, which is essential for normalization of the pressure in the medullary cavity.

METHODS

Experiments were carried out on chinchilla rabbits weighing 2 kg, not more than 2.5 months old. Under sterile conditions, a burr hole 0.3 cm in diameter was drilled in the rabbit's tibia in the region of the metaphysis, through which all cells of the bone marrow were removed by a powerful jet of 0.5% procaine solution, injected into the medullary canal. A narrow transverse cut was then made (taking very great care of the periosteum, which was preserved at the edges of the bone wound) transperiosteally through the whole thickness of the cortical lamina of the middle part of the medullary canal of the diaphysis. This enabled normalization of the intraosseous circulation fluid. The operation was performed under general anesthesia. An intramuscular injection of 1 ml of 1% trimeperidine was given. The animals

Department of Morbid Anatomy, N. N. Priorov Central Research Institute of Traumatology and Orthopedics, Ministry of Health of the USSR. Department of Pathomorphology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 6, pp. 755-757, June, 1986. Original article submitted July 19, 1985.

 0007-4888/86/0006-0854$12.50

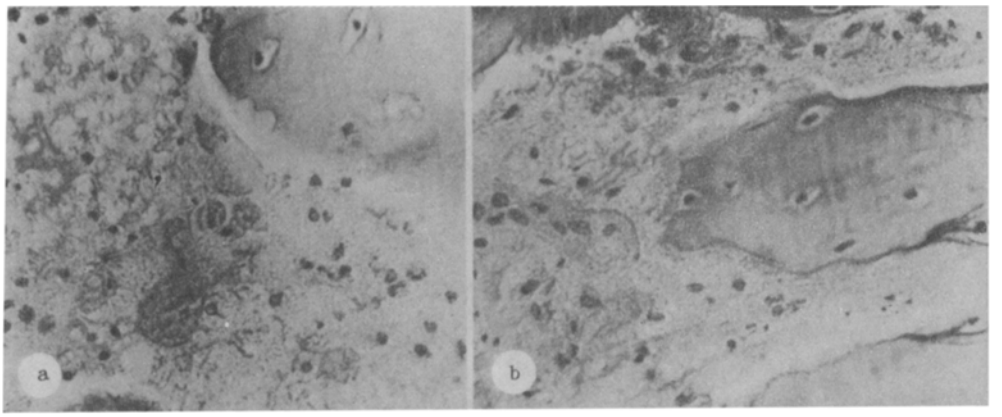

Fig. 1. Inner layer of cortical diaphysial lamina with dilated perforating Volkmann's canal, opening into medullary cavity. Undifferentiated connective-tissue cells can be seen in the lumen of the vascular canal. a) 2 days after curettage of bone marrow; b) 3 days after. Hematoxylin–eosin, 400×.

were taken from the experiment 1, 2, 3, and 6 days after the operation. Tissue for investigation was taken from the region of curettage. Histological and electron-microscopic autoradiographic investigations were undertaken. Pieces of tissue for histological study were fixed with 10% neutral formalin. Preparations were stained with hematoxylin and eosin and with picrofuchsine by Van Gieson's method. Pieces of tissue for electron-microscopic autoradiography were incubated in medium with ^{3}H-thymidine in a dose of 10 μCi/ml (specific activity 24 Ci/mmole) at 37°C for 1.5 h. The pieces of tissue were then carefully washed in cold medium 199, fixed, dehydrated, and embedded in Araldite. Type M photographic emulsion was applied to ultrathin sections intended for electron-microscopic autoradiography, by the method described previously [4, 5]. After exposure for 1 month the preparations were developed with D-19 developer and paraphenylenediamine, stained by Reynolds' method, and examined in the JEM-7A microscope.

RESULTS

On histological examination 24 h after the operation the medullary cavity was tightly packed with amorphous masses of plasma-like edema fluid, mixed with erythrocytes. Small concentrations of cells were seen in the lumen of the vessels forming an intraosseous vascular microcirculatory network, among the disintegrating blood-stained masses. In the medullary canal two days after the operation, a deposit was observed in the amorphous blood-stained masses with disintegrating erythrocytes, and areas of plasma were observed lying closer to the cortical lamina of the diaphysis, where there were solitary round cystic cavities. In the lumen of the overwhelming majority of vascular channels in the cortical lamina, cell-free masses were visible. Only in some perforating Volkmann's canals, located closer to the medullary canal, could round or oval cells with a large, dense nucleus be seen (Fig. 1). These cells could be identified as perivascular. Cells of the same character also were found in the lumen of several dilated Volkmann's canals, opening into the medullary canal. These cells in some places were expelled on to the inner surface of the cortical lamina of the diaphysis.

On the third day of regeneration vascular canals of the cortical lamina became larger, and the same cells could be seen there as after 2 days. Cells expelled into the medullary canal on the inner surface of the cortical lamina also were larger (Fig. 1). In the medullary canal itself there were significantly fewer masses of disintegrating erythrocytes, among which appeared many round cystic cavities filled with plasma-like fluid.

On the 6th day of regeneration the number of vascular canals of the cortical lamina increased, and the same cells could be identified in them as on the 2nd and 3rd day of regeneration. A region of concentration of cells could be seen in the medullary canal at the level of the incision in the cortical lamina, and about 200 cells could be counted in it. In the zone of the outlet for these cells into the medullary canal, an albuminous fluid and remnants of disintegrating erythrocytes could be seen. The remaining space of the medullary canal was occupied by cystic cavities of different sizes. Near the inner surface of the cortical lamina large cell concentrations, larger than in the earlier stages, also could be seen.

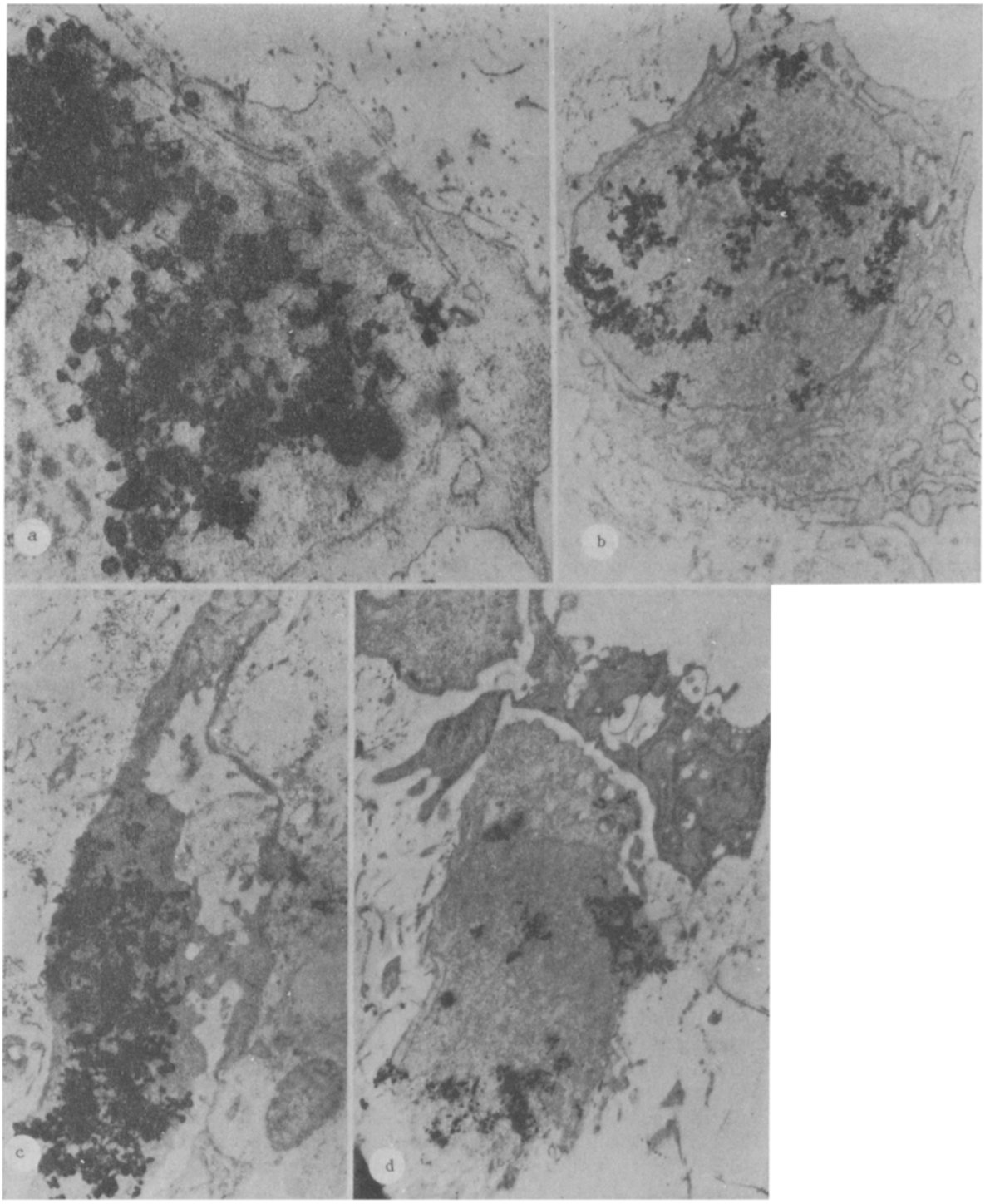

Fig. 2. DNA-synthesizing cells in regenerating bone marrow tissue 6 days after curettage of bone marrow. a) Undifferentiated connective tissue cell. 26,000×; b) differentiated osteogenic cell, 10,920×; c) endothelial cell, 8,580×; d) perivascular cell (pericyte). 9,620×.

The morphological pictures described above indicate the formation of intraosseous homeostasis and preservation of viability in cells of the vessel walls under conditions favorable for restoration of the circulation in the injured bone. Preservation of viability of the vascular cells also was manifested in their biological capacity — ability to proliferate. In all probability, the revival of these properties is attributable to the pericytes of vessels forming the microcirculatory network of vascular canals. The great resistance of cells of the vessel wall to a disturbance of their blood supply and nutrition of the microcirculatory network has been described in the literature [8].

To discover the proliferative properties of cells of the regenerating bone marrow tissue, an electron-microscopic autoradiographic investigation was carried out. This showed that the cell population which has the highest proliferative activity is morphologically heterogenous. It included in its composition cells with varied structural differentiation. Among them were undifferentiated and differentiated cells of connective-tissue type. The undifferentiated cells were small cells whose cytoplasmic membrane formed outgrowths of different sizes. Single, mainly flattened tubules of the rough endoplasmic reticulum, mitochondria, and an abundance of free ribosomes and polysomes were found in the cytoplasm. These undifferentiated connective-tissue cells were directly connected with blood vessels, but also lay separately from them. Successive development of elements of the rough endoplasmic reticulum and lamellar complex was observed in the differentiated cells. They were irregular or oval in shape. Their cytoplasmic membranes also was uneven and formed processes of different lengths. The cells described can evidently be regarded as a population of osteogenic cells. Thus DNA synthesis took place mainly in undifferentiated cells, endothelial cells, and differentiated cells (Fig. 2).

Since the main proliferating fraction among the DNA-synthesizing cells consists of undifferentiated connective-tissue cells, it can be tentatively suggested that they take part in types of histogenesis taking place in the regenerating bone marrow tissue, including in osteogenesis and angiogenesis. The results of this investigation agree with results of electron-microscopic autoradiography of biopsy material from regenerating tissue in Ilizarov's transosseous osteosynthesis [6] and with the results of electron-microscopic autoradiography of certain soft tissue tumors [7].

Perivascular undifferentiated connective-tissue cells of pericyte type, existing in Haversian and Volkmann's canals, thus evidently must be regarded as an inducible reserve. Various factors such as trauma, for example, may prove to be inducers of differential powers in one direction or another. Under the influence of changing local conditions and of the developing microenvironment, perivascular cells may be induced toward proliferation and differentiation into osteogenic cells. They can accordingly be regarded as inducible osteogenic precursor cells, which can take part in ectopic osteogenesis also.

LITERATURE CITED

1. M. A. Vorontsova and L. D. Liozner, Asexual Reproduction and Regeneration [in Russian], Moscow (1957).
2. A. A. Maksimov, Klin. Med. Wschr., 5, 2193 (1926).
3. A. V. Rumyantsev, Experience of the Study of Evolution of Cartilage and Bone Tissue [in Russian], Moscow (1958).
4. D. S. Sarkisov, Adaptive Reorganization of Biorhythms [in Russian], Moscow (1975).
5. D. S. Sarkisov, A. A. Pal'tsyn, and V. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
6. A. V. Smol'yannikov, Arkh. Patol., No. 2, 3 (1984).
7. D. S. Sarkisov, B. M. Kostyuchenok, Yu. A. Amiraslanova, et al., Arkh. Patol., No. 2, 17 (1985).
8. V. I. Stetsula, "Reparative regeneration of long bones in compression osteosynthesis," Dissertation for the degree of Doctor of Medical Sciences, Sverdlovsk (1965).
9. A. Ya. Fridenshtein and K. S. Lalykina, Induction of Bone Tissue and Osteogenic Precursor Cells [in Russian], Moscow (1973).