SELF-SUPPORT OF INDUCED BONE TISSUE

K. S. Lalykina, N. V. Latsinik,
S. Yu. Epikhina, and A. Ya. Fridenshtein

UDC 615.361.71

Ability of osteogenic precursors of bone marrow from intact and induced (by implantation of decalcified bone matrix) bones to support themselves was compared by transplantation in diffusion chambers. Osteogenesis continued for several months in bone marrow transplants from intact bones whereas in transplants from induced bone tissue active osteogenesis, although still observed after 12-20 days, ceased after 2 months. Fibroblasts from the second passage from cultures of hematopoietic tissue of induced bones, unlike fibroblasts from cultures of intact bone marrow, were virtually without osteogenic powers. These results confirm that induced osteogenic tissue has limited ability of self-support after the action of the inducer has ceased, compared with intact bone tissue.

KEY WORDS: induced bone tissue; self-support; hematopoietic stroma.

The possibility of induction of bone tissue in adult mammals is well known: Bone is formed and a microenvironment produced for the development of foci of myeloid hematopoiesis [6] around proliferating transitional epithelium [2, 11] or a decalcified bone matrix [14]. Induced osteogenic tissue can be kept for a long time (months) around autografts of the mucous membrane of the bladder or near zones of proliferation of transitional epithelium in the hilus of a kidney with its blood vessels ligated [12]. Meanwhile, bone induced by homografting of the mucous membrane of the bladder is absorbed within a few weeks [1]. After ligation of the renal vessels and autografting of the mucous membrane of the bladder, the transitional epithelium persists indefinitely at the site of induction; if homografted, however, it is absorbed by the end of the third to fourth week. Decalcified bone matrix, if implanted as inducer, is absorbed by the end of the third month and the bone tissue induced by it is also absorbed at the same time. It has accordingly been postulated that, unlike skeletal (determined) bone tissue, induced bone is preserved only as long as the inducing agent continues to act upon it [4], i.e., its maintenance is inducer dependent.

TABLE 1. Osteogenic Powers of Fragments of Hematopoietic Tissue from Foci of Bone Induction

Time of cultivation in chambers (in days)	Number of chambers	State of osteogenic tissue in chambers
12	4	Active osteogenesis. Extensive areas of osteoblasts and preosteoblasts seen around calcified bone lamellas spread over the surface of the filter and closely adherent to it
18	2	Ditto
20	1	"
65	2	Large quantity of mature calcified bone tissue, joining the two filters together, but with no osteoblastic layer. Bone cavities contain no cells or cells with signs of pycnosis. No recent osteogenesis
100	3	Ditto

The object of this investigation was to test this hypothesis. The degree of self-support of induced and determined osteogenic cells was assessed under tissue-culture conditions.

EXPERIMENTAL METHOD

Bone was induced by implantation of decalcified bone matrix into the anterior abdominal wall of adult rabbits. The inducing matrix was prepared from the femora of adult rabbits [14].

N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 239-242, February, 1976. Original article submitted July 24, 1975.

TABLE 2. Osteogenic Powers of Fibroblasts from Second Passage Cultures Grown from Hematopoietic Tissue of Pelvic and Induced Bones

Source of hematopoietic cells for cultures	Number of fibroblasts per chamber	Time of cultivation in chamber (in days)	No. of chambers	State of osteogenic tissue in chambers
Number of chambers	5×10^6	60	4	Large foci of active osteogenesis with peripheral zone of differentiating osteoblasts. Bone joins both filters together
Induced bone	5×10^6	60	4	Layers of connective-tissue cells with tiny fragments of dead bone, with no osteocytes or osteoblastic layer

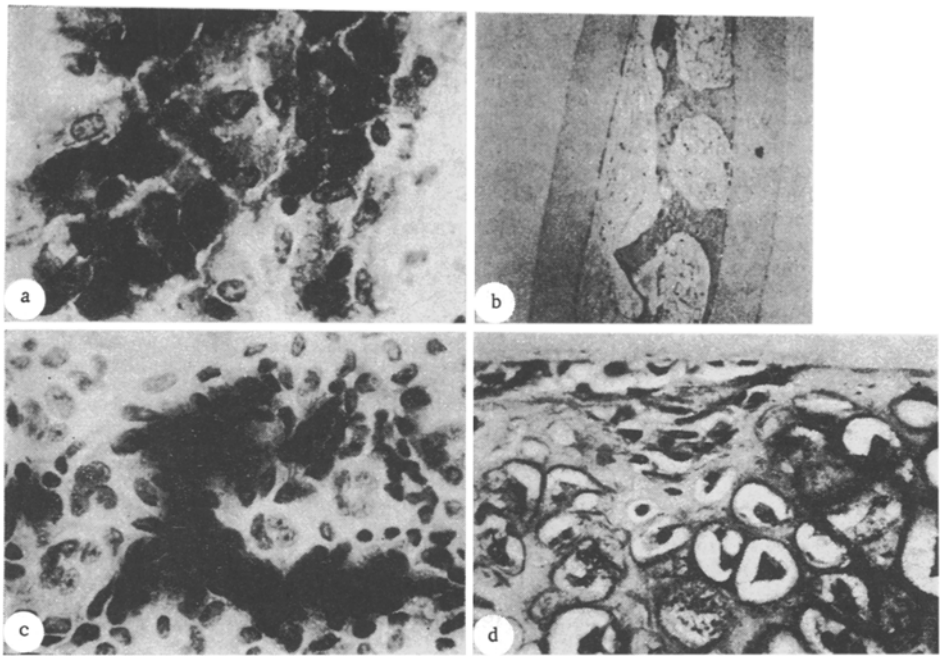

Fig. 1. Osteogenesis in diffusion chambers (Gomori's reaction for alkaline phosphatase, hematoxylin): a) osteogenic tissue in chambers with bone marrow from induced bones, 18 days (objective 20); b) dead bone tissue in chambers with bone marrow from induced bones, 65 days (objective 3); c) osteogenic tissue in chambers with fibroblasts from cultures of pelvic bone marrow, 60 days (objective 20); d) foci of cartilage tissue in chambers (c) (objective 40).

After 2-3 months, by separating the laminae of induced bone under a binocular loupe, the bone marrow was extracted and placed either (a) in diffusion chambers made from HA Millipore filters with a pore diameter of 0.45 μ, or (b) in monolayer cultures in medium No. 199 with 20% homologous serum, the cell suspension being prepared as described previously [9]. Bone marrow cells from the pelvic bones of the same rabbits were explanted in a parallel series of experiments.

On the 10th day the number of colonies of fibroblasts was counted in the cultures, and fibroblasts were removed from a parallel series of flasks, subjected to two passages, and then placed in diffusion chambers, which were implanted intraperitoneally into allogeneic rabbits. The chambers were removed and dismantled 12-100 days later, their flaps were fixed with 96° alcohol in the cold, and Gomori's test for alkaline phosphatase with counterstaining with alum-hematoxylin was carried out.

EXPERIMENTAL RESULTS

Reticular tissue with hematopoietic cells was distributed in the foci of induction between calcified bone lamellae, arranged along the implanted matrix. The results of transplantation of this tissue in diffusion chambers are given in Table 1.

On the average about 10^7 cells could be washed out of one focus of induced osteogenesis, and of the total number 50% were erythroid, 23% myeloid, 9% lymphoid, and 11% stromal cells. The mean cloning efficiency of these cells in monolayer cultures ($5.4/10^5$ cells) was rather higher than for cells of the pelvic bones ($3.5/10^5$ cells). This correlates well with the fact that the induced bone marrow was rich in reticular cells. The results of transplantation of fibroblasts from cultures of pelvic and induced bones in diffusion chambers are given in Table 2.

Bone marrow from skeletal bones contains osteogenic precursor cells histogenetically unconnected with hematopoietic stem cells [13]; because of the presence of the former, heterotopic transplantation of bone marrow [3] or its transplantation in diffusion chambers [7] leads to bone formation. In primary monolayer cultures its osteogeneic precursors form colonies consisting of clones of fibroblasts which, on reimplantation in vivo as diploid strains, having passed through more than 20 passages, exhibit strong osteogenic properties [5]. After removal by adhesion of cells from which colonies of fibroblasts were formed, the osteogenic powers of the bone marrow disappeared. These facts show that fibroblasts growing in bone marrow cultures are determined osteogenic precursor cells, capable of supporting themselves for a long time, and whose osteogenic powers are inducer independent.

This investigation shows (Fig. 1) that the medullary cavities of induced bones also contain osteogenic cells which can maintain osteogenesis in the chamber for not less than 18 days independently of contact with the inducer (which could not enter the chamber). However, toward the end of the 2nd month, osteogenesis ceased in the chambers and only mature bone remained. Conversely, in chambers with bone marrow from skeletal bones active osteogenesis continued for many months [10]. Whereas the freshly isolated osteogenic precursors from induced bone were capable of osteogenesis, even though to a limited degree, their progeny, in the form of second passage fibroblasts, were virtually without osteogenic powers.

Compared with skeletal bone tissue, induced osteogenic tissue in fact thus has limited ability to support itself after the action of the inducer has ceased. However, the reasons for this behavior is uncertain. Inducible osteogenic precursor cells (IOPC) can be eliminated from the populations as being capable of only a limited number of divisions. This would have to be the case if the IOPC were half-stem cells or if, as a result of induction, osteogenic cells with half-stem properties were formed from stem cells. On the other hand, loss of osteogenic powers in the population of induced cells could be connected with the loss of their osteogenic properties, caused by the action of the inducer, rather than with the cessation of existence of particular cell lines.

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