MORPHOLOGY OF THE INDUCTION PROCESS AFTER IMPLANTATION OF BONE MATRIX INTO MICE

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After implantation of decalcified bone matrix into mice bone with bone marrow is induced [1, 5-8]. However, the formation of an organ composed of bone and bone marrow is not the inevitable result of the action of the induction stimulus, and morphological pictures of the process of induction of bone formation vary in different recipients [1, 7, 8].

The object of this investigation was to study the suitability of induced bone and stroma for repopulation by hematopoietic cells. Bone matrix was implanted into normal and irradiated mice and also into animals after curettage of the femoral medullary canal. The morphology of the induction process was studied 1-2 months after implantation of the matrix.

EXPERIMENTAL METHOD

Decalcified bone matrix was prepared by Urist's scheme [5] from the tibias of (CBA × C57BL)F_1 mice. The matrix was implanted into syngeneic recipient mice immediately after preparation. It was grafted into the anterior abdominal wall of normal mice, mice irradiated with a dose of 450 rads, and mice subjected to curettage of the medullary canal of one femur 1 h before implantation of the matrix, and also beneath the capsule of the left kidney of normal mice. The implant with surrounding tissues was removed after 30-60 days and fixed with alcohol-formol or Zenker-formol by Maximow's method. Histological sections 5-7 μ thick were stained with hematoxylin-eosin, by the PAS method, and by the May–Gruenwald method and counterstained with azure-eosin.

EXPERIMENTAL RESULTS

Bone formation was induced in all series of experiments on average in 80% of cases. Induced bone differed in individual recipients depending on the degree of maturity and the degree of repopulation by hematopoietic cells. Morphologically, all the implants could be divided into four types: 1) implants with induction of laminae of cartilage or bone not containing a medullary cavity; 2) implants with induction of mature bone tissue without an osteoblastic layer, with a cavity containing a few hematopoietic cells; 3) implants with induction of bone tissue, lined by an osteoblastic layer, with a cavity filled with intensively hematopoietic bone marrow; 4) implants containing fragments of mature bone tissue with a cavity filled with infrequent hematopoietic cells, and fragments of young bone, lined by an osteoblastic layer and repopulated with hematopoietic cells. In all series of experiments except that in which matrix was implanted into animals subjected to curettage of the medullary canal, four types of induced bone were found. In the mice with a curetted femur only implants of type 2 were observed; altogether 42 implants were studied, 34 of them with osteogenic induction.

In the 11 type 1 implants laminae of cartilage or bone tissue were induced. The laminae contained from 10 to 100 cells and were located either within the implanted matrix or on its outer surface, in direct contact with muscle tissue. Cells forming the laminae were in one stage of differentiation, but sometimes foci with cells of different degrees of maturity were found. Occasionally several foci of chondrogenesis were present in the same implant of matrix. Mature bone tissue, which in some cases occupied the whole previous medullary canal of the implanted matrix, was formed in seven type 2 implants (Fig. 1). This bone had no osteoblastic layer and contained many lacunae with remnants of osteocytes. A cavity of considerable size, with fusiform

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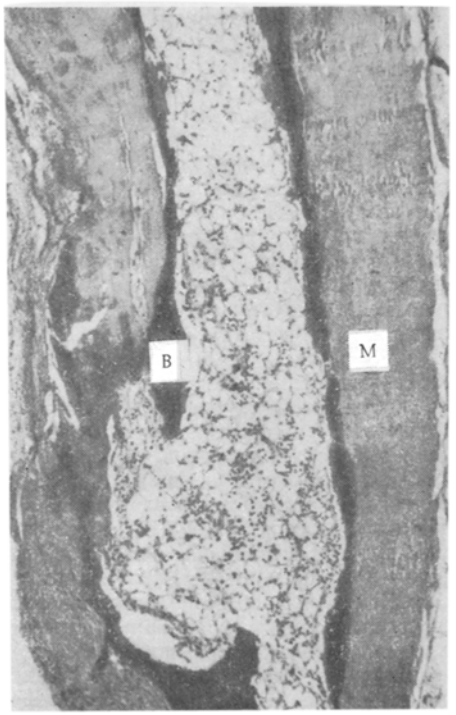

Fig. 1

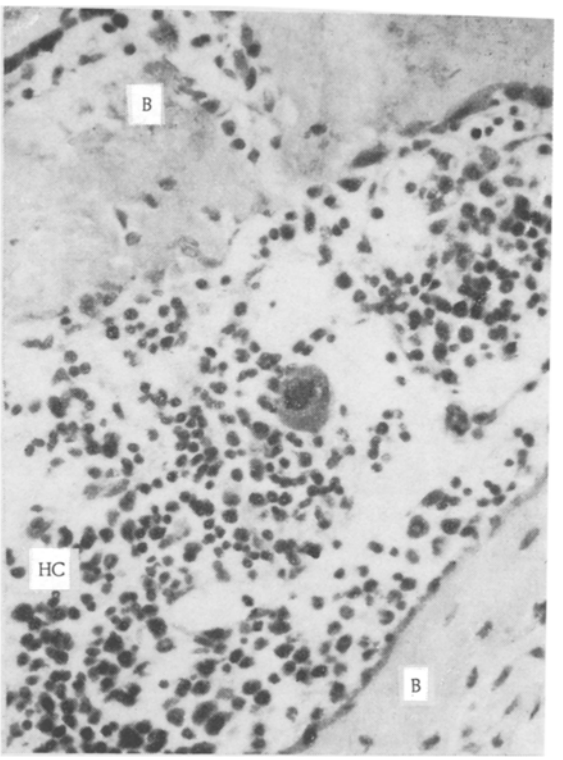

Fig. 2

Fig. 1. Induction of mature bone without bone marrow 40 days after intramuscular implantation of matrix into an animal subjected to curettage of the femoral medullary canal. M) Bone matrix; B) induced bone. PAS method, 25×.

Fig. 2. Induction of hematopoietic marrow 30 days after intramuscular inplantation of matrix. HC) Hematopoietic cells; B) induced bone. Hematoxylin-eosin, 200×.

reticular cells, forming a net-like structure in the meshes of which myelocytes, macrophagal cells, and lymphocytes could be seen, was formed within the induced bone. In some implants regions of mature bone and cartilage tissue were undergoing intensive resorption by osteoclasts. In 11 type 3 implants a bony capsule with a well-defined osteoblastic layer facing the medullary canal, which contained intensively hematopoietic marrow, was formed inside the matrix (Fig. 2). In the induced bone marrow with well-marked sinusoids there were cells which belonged to all branches of hematopoiesis: erythroid, myeloid, and megakaryocytic. The quantity of induced bone marrow in some cases was so great that the bony capsule could not envelop the whole of the hematopoietic tissue; however, the reticular stroma of the induced medullary organ was always separated from the net-like structure within the old matrix by layers of connective-tissue cells. In five type 4 implants both types of induced osteogenic tissue were found: young osteogenic tissue with an osteoblastic layer and a cavity repopulated with hematopoietic cells (Fig. 3), and mature bone without osteoblasts, with a smooth, polished surface facing inside the cavity, which contained isolated mature hematopoietic cells. Reticular cells, erythrocytes, and individual lymphocytes were found in the cavities repopulated by hematopoietic cells.

The morphological pictures of ectopic bone formation described above are evidence of different directions in the course of the induction process. The recipient's response to the induction stimulus may be restricted to the formation of foci of chondrogenesis and of individual bone fragments. During the formation of lamellar bone with a cavity, the question of the normality of the induced stroma for repopulation by hematopoietic cells must be considered. During induction it is evidently possible for three types of bony framework to be formed, one of which is constantly self-maintained through the active osteoblastic layer and is repopulated by the hematopoietic bone marrow, whereas the other is represented by mature bone tissue with depopulated stroma. In the case of induction of a medullary organ, all branches of hematopoiesis are observed after 1-2 months in the induced bone marrow. The induced bone marrow differs in no respects whatever from hematopoietic femoral marrow, in agreement with data obtained after implantation of bone matrix into rats and rabbits [4], the wall of the urinary bladder into guinea pigs and dogs [2, 3], and WISH cells into mice [9, 10]. The early

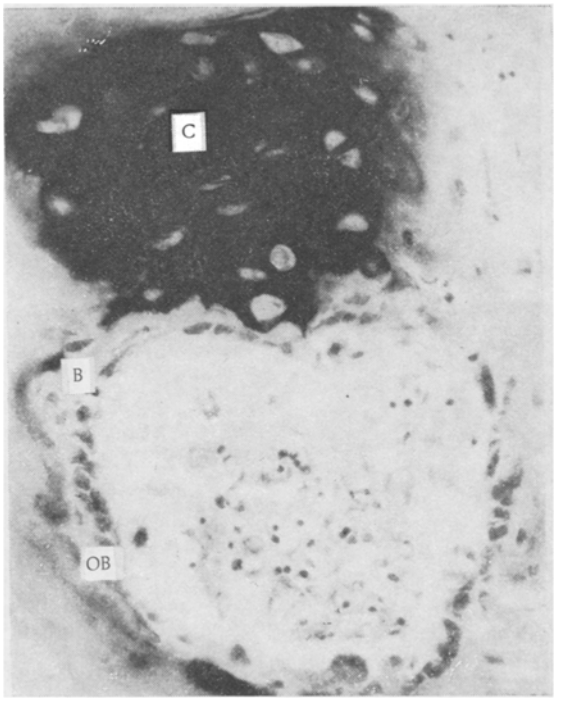

Fig. 3. Induction of bone 30 days after intramuscular implantation of matrix: young osteogenic tissue with well-marked osteoblastic layer and with a medullary cavity is repopulated by hematopoietic cells. C) Chondrogenesis; OB) osteoblastic layer; B) induced bone. Stained by May–Gruenwald and azure-eosin methods; 200×.

stages of the induction process, leading to the formation of a medullary organ, are interesting. Reticular cells, erythrocytes, and lymphocytes were found inside the bony cavities lined with osteoblasts. Spellman et al. [3], who studied the morphology of the induction process after transplantation of transitional epithelium of the urinary bladder in dogs, also observed the appearance of cavities with erythroid cells and lymphocytes initially in the induced bone foci, followed by myelopoiesis and megakaryocytes. It is important to note that the stroma of the induced bone marrow was never in contact with the reticular structure of the matrix and that the induced bone marrow was always separated from the net-like structure of the matrix by layers of connective tissue.

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