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TRANSFER OF BONE-MARROW MICROENVIRONMENT BY CLONES OF STROMAL MECHANOCYTES

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Colonies consisting of clones of bone-marrow stromal fibroblasts, grown in monolayer cultures of mouse and guinea pig bone-marrow cells, transfer the hematopoietic microenvironment on retransplantation into the animal. Individual clones simultaneously form bone tissue and create a microenvironment for all three branches of medullary hematopoiesis: erythroid, myeloid, and megakaryocytic.

KEY WORDS: bone marrow, hematopoietic microenvironment.

The writers showed previously [6] that the formation of a new hematopoietic organ at the site of heterotopic transplantation of bone marrow takes place as a result of survival of stromal mechanocytes, which form a territory for colonization by hematopoietic cells, i.e., they create a hematopoietic microenvironment. Stromal mechanocytes are local, and not repopulating cells: In the hematopoietic tissue of radiochimeras they preserve their recipient origin, whereas in heterotopic grafts they remain of donor origin [8]. The hematopoietic organs contain clonogenic stromal precursors, which can be detected by the formation of colonies consisting of clones of fibroblasts in cultures [3]. During subculture of these cultures diploid strains of fibroblasts arise and, if retransplanted into the animal, they transfer the hematopoietic microenvironment [6]. The question arises whether individual clones of stromal mechanocytes can transfer the bone-marrow microenvironment or whether cooperation between several different clonogenic stromal precursors is required for this purpose. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Bone marrow cells from adult CBA mice and guinea pigs were used for cloning bone-marrow mechanocytes [5, 7]. Mouse bone marrow was extracted from the femora of the killed donors; guinea pig bone marrow cells were flushed out of the femur by means of a needle through the distal epiphysis of the anesthetized animal, after compression of the femoral artery. Cell suspensions were filtered through four layers of nylon and explanted into Roux flasks, the bottom of which was first covered with collagen gel. Guinea pig cells were cultured in medium No. 199 with 20% bovine serum, mouse cells in Fisher's medium with 15% embryonic serum. On the 16th-30th day colonies of fibroblasts together with the collagen gel were cut out and transplanted into animals. Colonies of mouse cells were transplanted into syngeneic recipients beneath the capsule of the kidney; colonies of guinea pig cells were autografted by introducing them in to the diaphysis of a homologous femur, freed from bone marrow and irradiated in a dose of 5000 R, which was then implanted into the muscle of the anterior abdominal wall. In control experiments collagen gel was implanted beneath the capsule of the kidney and irradiated cylinders of bone without colonies of fibroblasts were implanted into the abdominal muscle. After 30-90 days the kidneys and the bone cylinders were fixed in alcohol and formol, decalcified,

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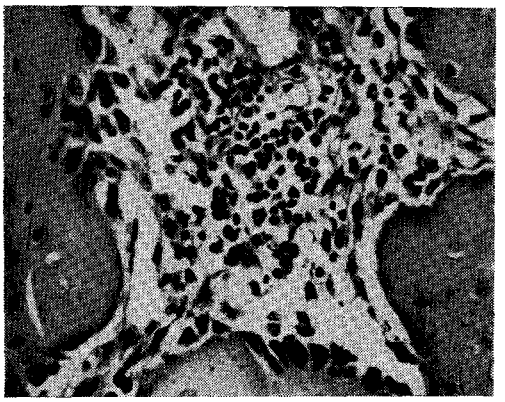

Fig. 1. Focus of bone and hematopoietic tissue at site of transplantation of colonies of guinea pig bone-marrow fibroblasts. Time of investigation 50 days. Objective 20 ×.

and embedded in paraffin wax; series of sections were stained with hematoxylin-eosin and azure-eosin and the Hotchkiss reaction was carried out.

EXPERIMENTAL RESULTS

In cultures of collagen gel colonies of fibroblasts formed by the 8th-10th day in the same way as was described previously for monolayer cultures of bone marrow cells on glass [3, 5-7]. Colonies of guinea pig cells were contaminated with a very few macrophages or they consisted entirely of fibroblasts, whereas in cultures of mouse bone marrow contamination with macrophages was considerable.

The results of transplantation of colonies of bone-marrow fibroblasts were essentially as follows. In the control experiments the cavities of the irradiated bone cylinders were filled with connective tissue with no evidence of osteogenesis or hematopoiesis; the walls of the cylinders were thin and contained no osteocytes. In the kidneys, at the site of control transplantation very slight thickening of the capsule was present or no changes were observed whatsoever. The same results, indistinguishable from the control, were obtained at the site of transplantation of about 80% of the fibroblast colonies. Otherwise transplantation of the colonies led to the development of bone tissue alone or bone and hematopoietic tissue.

The most interesting discoveries were bony foci with a medullary cavity filled with bone marrow. These foci inside the bone cylinders were round in shape and had a capsule composed of bone tissue with osteocytes and a well-developed layer of osteoblasts. The medullary cavity contained reticular tissue with myeloid and erythroid cells (Fig. 1). In the kidney the bony capsule covered a medullary cavity only on its outer surface. Hematopoietic cells (about $4 \cdot 10^5$ per graft) consisted of myeloid or erythroid cells and megakaryocytes (Figs. 2 and 3). At the site of transplantation of some of the colonies beneath the kidney capsule, bone tissue with no trace of hematopoiesis was found. Some of these foci consisted of compact bone tissue with osteocytes and an incomplete layer of osteoblasts; in other cases the newly formed bone tissue was already dead at the moment of fixation, i.e., it contained no osteocytes and no layer of osteoblasts.

The formation of a new hematopoietic organ after transplantation of bone marrow was determined by survival of the stromal and not of the hematopoietic cells or macrophages. This is clear from the results of transplantation of bone marrow of radiochimeras into animals of the donor's and recipient's strain [4] and transplantation of bone marrow from heterotopic grafts [2]. All stromal mechanocytes and their precursors preserve their donor's origin in heterotopic grafts [8]. For that reason, the formation of hematopoietic tissue on transplantation of stromal cells can serve as a test of their ability to transfer the hematopoietic microenvironment, and that contamination of colonies of stromal mechanocytes with macrophages does not prevent the stromal colonies from being regarded as carriers of the microenvironment. Evidence that the cells forming colonies in monolayer cultures of bone marrow are indeed mechanocytes was given by their morphological features, by the synthesis of collagen by these cells, and by the presence of surface receptors and antigens specific for fibroblasts but not for hematopoietic cells or macrophages on them [1]. The clonal nature of the colonies was demonstrated by chromosomal markers, by intravital motion picture filming and by [^{3}H]-thymidine labeling [1, 3]. The results confirmed the osteogenic powers of stromal mechanocytes grown in culture [3] and also showed that the hematopoietic microenvironment can be transferred by their transplantation.

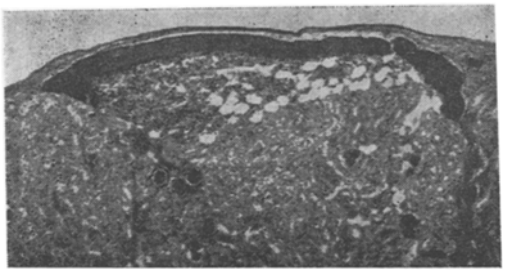

Fig. 2

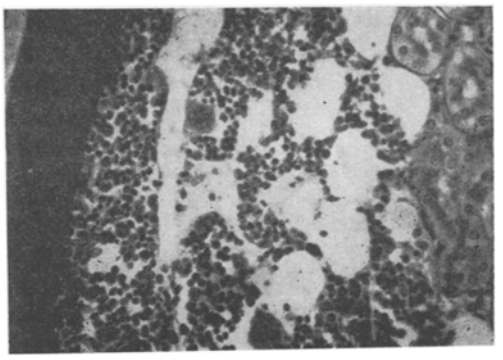

Fig. 3

Fig. 2. Focus of bone tissue with bone marrow at site of transplantation of colony of mouse bone-marrow fibroblasts. Time of investigation 90 days. Objective 20 ×.

Fig. 3. The same section, objective 40 ×.

The main result of the investigation was to establish the fact that individual clones of stromal cells and, consequently, individual bone-marrow stromal precursors can create a hematopoietic microenvironment for all three branches of hematopoiesis at the same time: erythroid, myeloid, and megakaryocytic. It is too early as yet to judge the order of precedence of the clonogenic cells forming nonself-supporting (dying in the grafts) bone tissue and cells forming bone capable of supporting itself for a long time and, at the same time, transferring the microenvironment for hematopoiesis. Nor is it known to what degree the processes taking place during development of clones influence their ability to transfer the microenvironment during subsequent transplantation. For all these reasons it is impossible as yet to assess what proportion of all clonogenic stromal cells can transfer the cellular microenvironment, but the possibility cannot be ruled out that it is in fact low, and that there are only a few stromal cells with the properties of stem cells.

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