Bone Marrow Repopulation of Subcutaneously Grafted Mouse Femurs[1] (35938)

SHELDON AMSEL AND ELISABETH S. DELL
(Introduced by H. J. Zimmerman)
Departments of Medicine and Nuclear Medicine, Boston Veterans Administration Hospital, Boston, Massachusetts 02130

Following the subcutaneous syngeneic grafting of bone marrow or bone containing marrow in its medullary cavity the following sequence of histologic events occurs: disappearance of bone marrow cells, proliferation of primitive appearing "preosteoblasts," cancellous bone formation, reappearance of marrow cells and cancellous bone resorption (1, 4). In either instance the histologically stable implant is a small ossicle containing sinusoidal hemopoietic tissue. The reappearance of hemopoietic cells in the evolving implant raises the question of whether they are derived from the transplanted donor tissue (*i.e.*, produced locally) or whether they are derived from the stem cells of the recipient which have migrated into the ossicle and proliferated (1, 2). To answer this, syngeneic subcutaneous transplants of bone marrow and femurs between CBA/CA and CBA/H-T6 mice were performed. These strains are histocompatible; however, the latter strain is homozygous for a chromosomal translocation making their chromosomes in metaphase readily distinguishable (9). Two hundred days following the above grafts the hemopoietic cells in the subcutaneous ossicles were prepared and recorded as being derived from the "CA" or "T6" line.

Materials and Methods. Female CBA/CA and CBA/H-T6 mice (Jackson Memorial Laboratories, Bar Harbor, Maine), weighing approximately 25 g, were used in all experiments. Unless otherwise noted, the words transplant or graft connote the syngeneic grafting of hemopoietic tissue or femur of a donor mouse to a subcutaneous site in the recipient mouse.

[1] Supported by U.S. Public Health Service Grant 5 R01 AM 13723-02.

Bone marrow transplants. Five CBA/CA mice were killed by cervical dislocation, the femurs were removed, and the epiphyses were transected. A 25 gauge needle was placed in the shaft and the marrow was air-blown onto a moistened scalpel blade. The marrow was removed from the femurs of one mouse before killing the next. A recipient CBA/H-T6 mouse was anesthetized and a midline abdominal skin incision was made. By pushing a hemostat laterally a narrow subcutaneous pocket was created. The cohesive mass of hemopoietic tissue from 10 femurs was then pushed off the scalpel blade and into the pocket with a hemostat and the incision was closed. Two of the above transplants were done, plus two more reversing the recipient and donor. After 200 days the four recipients were killed and their subcutaneous ossicles were removed. These were rigid bony spicules measuring roughly 6 $\times$ 1 mm. The ends of the spicule were clipped off with a scissors and the cells were flushed into an incubating solution (TC199, Difco) using a TB needle and syringe and incubating solution. After 1 hr at 37°, colchicine was added to make a 10^{-5} *M* solution. After 2 hr more at 37°, metaphase plates were prepared using the method of Kiossoglou *et al.* (3).

Femur transplants. Eleven donor CBA/CA mice were killed and the shafts of their 22 femurs were placed in the abdominal subcutaneous space of 11 recipient CBA/H-T6 mice (two femurs to a recipient). After 200 days the femurs, measuring 12 $\times$ 2 mm, were removed and their contents were flushed into the incubating solution as described above. The marrow from two subcutaneous ossicles was flushed into one incubating solution and considered as one transplant. The

experiment was repeated using 10 CBA/H-T6 mice as the donors and 10 CBA/CA mice as the recipients.

Results. The cells harvested from the 4 ossicles formed after transplanting hemopoietic tissue were few in number and no cells in metaphase were detected. In general, the cells harvested from the subcutaneous "femurs" were greater in number. Of the 21 "femur" transplants, 14 had cells in metaphase and a total of 102 metaphase plates were counted. Five of the 14 transplants had metaphase plates of only one type, that of the recipient. The remaining 9 transplants were chimeras, yielding 76 metaphase plates of which 47 were derived from the donor and 29 from the recipient. H and E sections of ossicles formed after transplantation of bone marrow or bone containing marrow have previously been demonstrated (1, 4).

Discussion. The osteoblasts which form cancellous bone following the syngeneic transplantation of hemopoietic tissue to a subcutaneous site are derived from cells in the donor hemopoietic tissue (2). The osteoblasts which form cancellous bone following the transplantation of a femur can theoretically originate from three sources: the contained hemopoietic tissue (2), the periosteal and endosteal osteocytes of the femur (5), and they can be induced to differentiate from the recipient's surrounding mesenchymal tissue cells by the grafted bone (6). Thus after transplanting a femur, the cellular elements of the bone of the subcutaneous ossicle may be both of donor and host origin.

It is thought that marrow sinusoids are constructed in the interstices of this newly formed cancellous bone (7). It is unclear which cells accomplish this, but they probably are, like the osteoblasts, derived from the transplanted donor tissue (1, 7). The repopulation of the subcutaneous implant with bone marrow cells becomes evident after the sinusoidal circulation is complete (1). If the same cells which give rise to the osteoblasts and sinusoidal cells also differentiated to form hemopoietic stem cells this could then be the mechanism for local production of stem cells. It would also explain our findings of chimerism, since, as mentioned above, the cellular elements of the bone may be chimeric themselves. Experimental evidence that hemopoietic stem cells can be produced locally was provided by Fong *et al.* (8) following the mechanical depletion of cells in the mouse femur *in vivo*. The cells found in the repopulated femur were shown to be derived from cells of that femur and did not migrate from other areas of bone marrow. This experiment, like ours, suggests that hemopoietic cells proliferating in a bone which was previously depleted of cells can differentiate from locally produced stem cells.

The data of Friedenstein *et al.* (2) are at variance with ours and the above interpretation. Also using the T6 chromosome marker, hemopoietic tissue was grafted heterotopically and the subcutaneous ossicles were removed after 14 months. Of 52 metaphase plates obtained from the marrow cells flushed from three ossicles, all were of recipient origin. The authors concluded that the heterotopic ossicle is repopulated by hemopoietic stem cells of the recipient which migrated through the blood. The possible causes for their finding only metaphase plates of the recipient are that not enough transplants were performed or that the different donor material which was used proved to be significant (bone marrow vs femur containing its bone marrow). Our finding of recipient's cells in all 14 implants which contained metaphases suggests that even though local production of donor and recipient cells may be occurring, repopulation by migration of recipient's stem cells cannot be excluded as a simultaneous event. Indeed, it seems reasonable to conclude that both processes are occurring at the same time.

Another possible explanation for chimerism in the heterotopic ossicle is that the disappearance of the donor hemopoietic cells in the freshly transplanted femur (see introduction) involved migration of the hemopoietic stem cells of that femur into the recipient. This would produce two populations of stem cells in the recipient. When the subcutaneous ossicle evolved histologically to the point where it could support hemopoietic tissue, and if repopulation took place by stem cell migration into the graft, a chimera would be

produced. This possibility appears unlikely since the donor stem cells would only be a small fraction of the recipient's stem cell pool and yet 46% of the 102 cells in metaphase found in the ossicles were of donor karyotype.

Summary. Using the T6 chromosome marker, the karyotypes of hemopoietic cells which repopulated subcutaneously transplanted femurs in syngeneic mice were observed 200 days after transplantation. Nine ossicles contained cells derived from both donor and recipient and 5 contained cells derived only from the recipient. It is postulated that the cellular elements of the new bone, which is formed after transplantation, contains cells derived from both donor and recipient. It is further suggested that these cells can differentiate to form hemopoietic cells thus explaining the presence of chimeras in the ossicles. The migration of the recipient's hemopoietic stem cells into the ossicle with subsequent proliferation probably occurs simultaneously.

The authors are indebted to Mrs. Nanci Coleman for her skill in preparing the chromosome karyotypes.

1. Tavassoli, M., and Crosby, W. H., Science **161**, 54 (1968).

2. Friedenstein, A. J., Petrakova, K. V., Kurdlesova, A. I., and Frolova, G. P., Transplantation **6**, 230 (1968).

3. Kiossoglou, K. A., Mitus, W. J., and Dameshek, W., Amer. J. Clin. Pathol. **41**, 183 (1964).

4. Burwell, R. G., J. Bone Joint Surg. (Brit.) **46**, 110 (1964).

5. DeBruyn, P. P. N., and Kabisch, W. T., J. Anat. **96**, 375 (1955).

6. Urist, M. R., Dowell, T. A., Hay, P. H., and Strates, B. S., Clin. Orthop. **59**, 59 (1968).

7. Amsel, S., Maniatis, A., Tavassoli, M., and Crosby, W. H., Anat. Rec. **164**, 101 (1969).

8. Fong, P. L., Maloney, M. A., and Patt, H. M., Blood **37**, 413 (1971).

9. Ford, C. E., and Micklem, H. S., Lancet **1**, 359 (1963).

Received June 7, 1971. P.S.E.B.M., 1971, Vol. 138.