Osteogenic Potential of Leukemic Marrow[1] (37328)

R. JUDITH RATZAN,[2] MEHDI TAVASSOLI,[3] AND WILLIAM H. CROSBY[3]

Blood Research Laboratory, New England Medical Center Hospitals and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

Osteogenic potential of marrow tissue has been well established (1–3). After mechanical injury or transplantation of marrow tissue to an ectopic site, primitive connective tissue cells (fibroblasts) appear which differentiate into osteoblasts and begin to lay down osteoid material which later calcifies (4). Using chromosomal analysis (5) and differential sensitivity to radiation (6), it has been shown that fixed reticular cells associated with stromal microcirculation of marrow are responsible for this osteogenic potential. Hematopoietic cells, *per se,* are not capable of bone formation. Recent electron microscopic studies on acute granulocytic leukemia in the rat have indicated the presence of injury to this reticulum meshwork (7). Present work was undertaken to see if this injury has eliminated the osteogenic potential of bone marrow tissue.

Materials and Methods. More than 150 white rats of Wistar–Lewis strain of both sexes were used in these experiments. They were obtained from the Charles River Breeding Laboratories. Although this colony had initially been derived from a truly inbred strain, the animals were subsequently bred randomly by the breeding laboratories. The Shay chloroleukemia tumor [courtesy of Drs. E. Handler (New York) and W. C. Moloney (Boston)] was maintained by subcutaneous passage of 40–80 million cells. To induce leukemia, a tumor of 2 to 3 wk of age was excised. A cell suspension was made by passing the tumor through tantalum gauze. The cells were then washed three times by centrifugation for 20 min at 800*g*. They were suspended in Hanks' balanced salt solution. Twenty million cells in 0.1 ml were injected into 50 g rats via a tail vein. Six or 8 days after induction of leukemia, hematocrit, white blood cells, reticulocyte and platelet counts were determined to evaluate the extent of the disease. The marrow tissue was then removed from these animals for implantation into the subcutaneous tissue of their littermates. The technique for extramedullary marrow implantation has been described in detail in several previous communications (8). Briefly, the knee joint was exposed and a hole was drilled into the articular surface of the femur. A polyethylene catheter (gauge 160) was driven gently into the marrow cavity for the whole length of the femoral shaft. The free end of the tube was then clamped and the tube was slowly removed. The marrow tissue which now filled the tube was implanted into a pocket prepared in subcutaneous tissue of the abdomen of a littermate animal. Normal marrow tissue, obtained in a similar fashion from a third littermate was implanted in the opposite side and this was used as a control. The leukemic animal was then killed and smears of bone marrow were prepared and stained with Wright–Giemsa stain. Tibia, spleen, and on occasion, liver and lung tissue were removed and fixed for histologic examination and assessment of leukemic involvement. Implants were removed at vary-

[1] These studies were aided with funds from U.S. Public Health Service Grant No. AM 12444- from the National Institute of Arthritis and Metabolic Diseases and Atomic Energy Commission Contract number AT (11-1) 3375.

[2] Trainee in Hematology under U.S. Public Health Service Graduate Training Grant No. AM 5210- from the National Institute of Arthritis and Metabolic Diseases. Present address: University of Miami, FL.

[3] Present address and reprint requests to: Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, CA 92037.

ing time intervals after implantation. They were evaluated for presence of bone, both grossly and microscopically.

Results. Two groups of animals were studied:

Group I (25 rats). The marrow tissue was removed and implanted 6 days after the intravenous injection of the animal by tumor cells. These animals showed anemia with a mean hematocrit of 37 vol %, mild leukocytosis and the presence of from 0–40% blasts on their peripheral smears with a mean of 6%. The marrow was uniformly infiltrated and even replaced by leukemic cells and there was almost uniform involvement of the spleen.

Group II (31 rats). The marrow tissue was used for implantation 8 days after the animal was injected intravenously with leukemic cells. These animals had far more profound anemia and peripheral smears showed from 14 to 100% blasts with a mean of 60%. Marrow (Fig. 1) and spleen were again infiltrated and sometimes completely replaced by leukemic cells. When examined, infiltration of liver and lung was also evident.

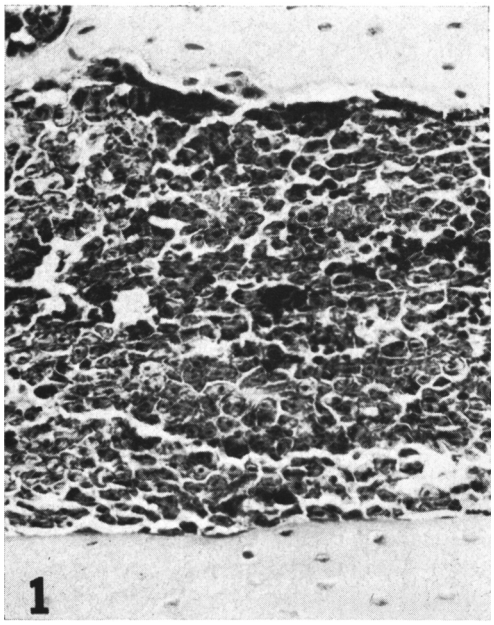

FIG. 1. Section of tibial marrow 8 days after induction of leukemia. Tubular bone is seen in upper and lower part of this figure. Leukemic cells have replaced the normal hemopoietic tissue. ×275.

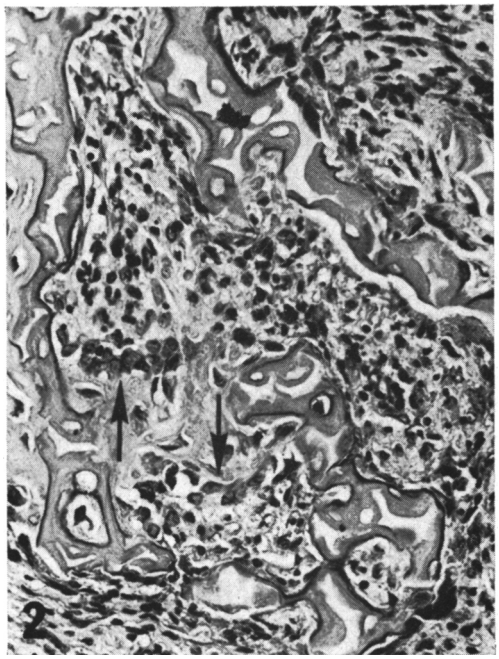

FIG. 2. Bone formation in implant of leukemic marrow. Note the osteoblasts lining the bone in several areas (arrows). Within the osteoid bone, infiltration of round cells is evident. This indicates the rejection of newly formed bone. Changes in staining characteristics of the osteoid substance and the lack of osteocytes in the lacunae are further evidence of rejection. Day 14 after implantation. ×275.

Subcutaneous implants of leukemic marrow developed in two different but distinctive pathways. In a small number of animals (3 and 6 rats in groups I and II, respectively) the implants resulted in growth of large subcutaneous tumors which, on microscopic examination consisted of leukemic cells. No marrow structure or bone was discernible. The tumor was usually large enough to prevent adequate evaluation of control marrow implant. However, when the implant of normal marrow was recovered, osteogenesis was observed in a fashion similar to other implants of normal marrow (vide infra). There was no correlation between the degree of marrow infiltration or the presence of leukemic cells in the blood and subsequent tumor formation.

When the implants of leukemic marrow

did not form tumor, *i.e.*, in the majority of cases, bone formation was observed uniformly when the implants were examined between 7 to 16 days after implantation (Fig. 2) and, in every case, the control implant of normal marrow also revealed bone formation (Fig. 3). No leukemic cells were observed in these implants.

On histological section of the implants on Day 7 after implantation, the bone appeared as trabecular in nature, the surface of which was lined with osteoblasts. Normal osteocytes were present within the lacunae. In the interstices of trabecular bone, however, round cell infiltration was evident (Figs. 2 and 3). In specimens removed at 12–16 days after implantation further evidence of rejection can be observed. This is characterized by lack of osteocytes within the lacunae and replacement of lining osteoblasts by fibrous tissue. The bony tissue loses its normal staining characteristic and often appears fragmented. Many foreign body giant cells are seen to be surrounding the fragments of bone. The rejection phenomenon proceeds further and by Day 35 when all remaining animals were examined, few, if any, bony spicules were present. Osteogenesis was similar, both qualitatively and quantitatively in normal and leukemic implants.

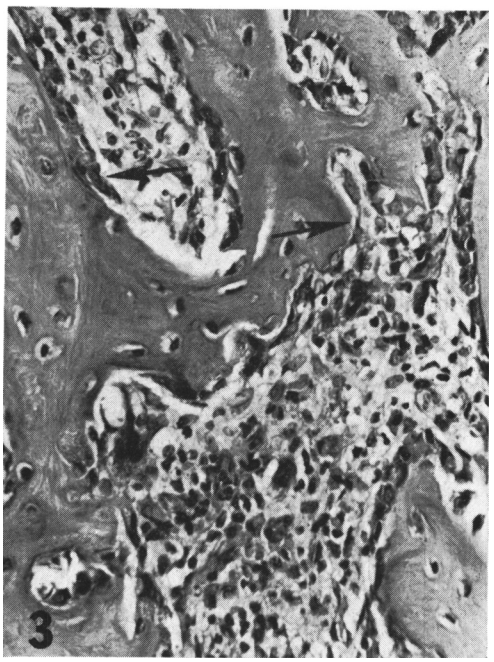

FIG. 3. Implant of normal marrow on day 14. Bone formation is again seen with osteoblasts lining the bone (arrows). The process of rejection is at a relatively earlier stage. Round cell infiltration in the interstices of osteoid tissue, however, is evident. ×275.

Discussion. Acute granulocytic leukemia induced in rats by intravenous injection of a cell suspension from Shay chloroleukemia tumor is a suitable model for studying the pathogenesis of this disease. The consistent and predictable course of the disease is associated with progressive infiltration of marrow and often spleen, followed by the appearance of leukemic cells in the peripheral blood. Some 12 days after induction of leukemia, almost all the animals died from the disease. Our observation confirmed this previously described course (9).

Implantation of leukemic marrow tissue to the subcutaneous sites resulted in formation of either bone or a mass of leukemic tissue. Bone formation in these implants indicates that the leukemia does not eliminate the osteogenic potential of the marrow. This occurs despite the injury to stromal microcirculation of the marrow (7) from which the osteogenic precursor cells have been shown to be derived. This observation may be of significance since granulocytic leukemias, in contrast to other infiltrative marrow disorders such as myeloma, rarely results in bone destruction.

The development of a mass of leukemic tissue in a few cases is of interest. When normal marrow tissue is similarly implanted, hemopoietic precursor cells disappear from the implant within 24–48 hr. They are either carried away into general circulation by rapidly ingrowing vascular sprouts or they undergo necrosis and phagocytosis (10). Clearly, this process also occurs in most implants of leukemic marrow since no leukemic cells were seen in those implants which formed bone. Leukemic cells appear to leave the implant and the remaining stroma gives rise to bone formation in a fashion similar to implants of normal marrow.

In a few cases, however, leukemic cells begin to proliferate and result in formation

of a solid tumor. This process then overrides the process of bone formation. Proliferation of leukemic cells in this situation appears to be similar to growth of chloroleukemia after subcutaneous injection of 30–40 $\times$ 10^6 leukemic cells (9). The factors determining which of the two processes (bone or tumor formation) may occur are not clear. Factors such as a critical mass of leukemic cells present in the implant, tumor angiogenic factor, the amount of C-type particles present and the immunologic status of the recipient may be of importance.

Rejection of the tissue which occurred in our experiment after 12–15 days is to be expected since the grafts were allogenic. The rejection phenomenon, however, occurred late enough to allow osteogenesis to develop in these implants.

Summary and Conclusions. Intravenous injection of 20 $\times$ 10^6 Shay chloroleukemia cells into 50 g rats induces acute granulocytic leukemia with infiltration of marrow and blood. Most animals die within 12 days.

Marrow tissue obtained from these animals and implanted into subcutaneous tissue of normal recipients produces bone which is later rejected. We, therefore, conclude that the leukemic marrow tissue, despite the reported injury to its potentially osteogenic cellular reticulum, retains its osteogenic potential. This observation may be of significance in explaining why the course of leukemia, unlike myeloma and other infiltrative diseases of the marrow, is not associated with bone destruction.

1. Pfeiffer, A., Anat. Rec. **102**, 225 (1948).

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

3. Williams, R. G., Anat. Rec. **129**, 187 (1957).

4. Tavassoli, M., and Weiss, L., Anat. Rec. **171**, 477 (1971).

5. Friedenstein, A. J., Petracova, K. U., Kurolesova, A. I., and Frolova, G. P., Transplantation **6**, 230 (1968).

6. Maniatis, A., Tavassoli, M., and Crosby, W. H., Blood **38**, 569 (1971).

7. Chen, L. T., Handler, E. E., Handler, E. S., and Weiss, L., Blood **39**, 99 (1972).

8. Tavassoli, M., Maniatis, A. K., and Crosby, W. H., Proc. Soc. Exp. Biol. Med. **133**, 878 (1970).

9. Handler, E. E., and Handler, E. S., *in* "Regulation of Hematopoiesis" (A. Gordon, ed.), Vol. 2, p. 1273. Appleton-Century-Crofts, New York (1970).

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

Received June 9, 1972. P.S.E.B.M., 1973, Vol. 143.