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Bone Resorption Restored in Osteopetrotic Mice by Transplants of Normal Bone Marrow and Spleen Cells

Abstract. *Capacity to resorb bone and calcified cartilage was restored permanently in mice with inherited osteopetrosis by the intravenous administration of cell suspensions prepared from spleen and bone marrow of normal littermates. Beginning near active growth plates as early as 2 weeks after transplantation, replacement of the abnormal spongiosa continued until medullary cavities were fully expanded.*

Capacity to resorb bone and calcified cartilage has been restored in mice (*1*) and rats (*2*) with inherited osteopetrosis through establishment of a cross-circulation between the affected animal and one of its normal littermates. A parabiotic union of only 1 or 2 weeks duration is long enough to initiate recovery, which progresses to completion and remains permanent after the cross-circulation is severed (*3*). Migratory cells representing precursors of competent osteoclasts or the source of a humoral factor essential to osteoclastic function were thought to mediate the recovery. In the investigation reported here sources of the therapeutically effective cells have been found in the normal bone marrow and spleen which, when infused into lethally irradiated osteopetrotic littermates, permanently restored capacity to resorb skeletal matrix.

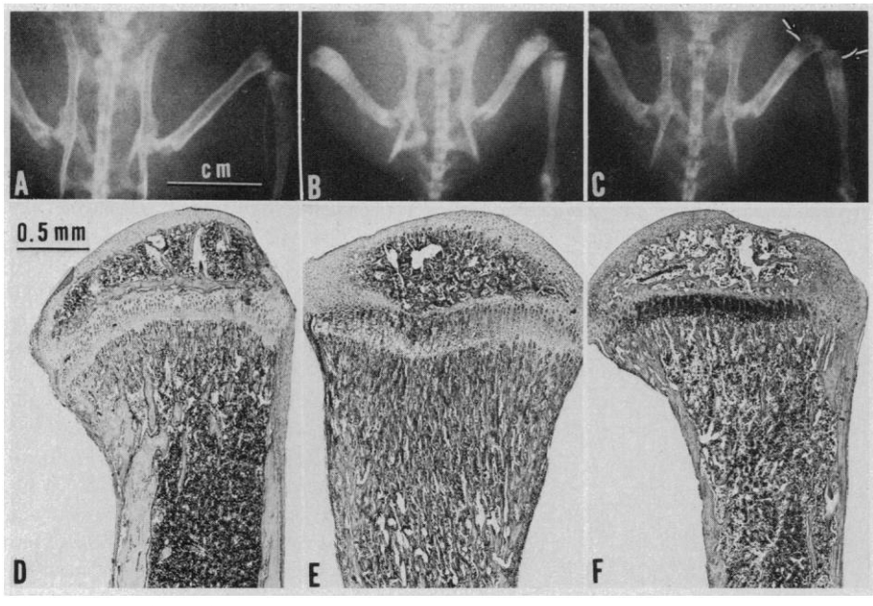

Fig. 1. (A to C) Radiograms (× 2) of normal control (A), osteopetrotic control (B), and osteopetrotic experimental (C) mice as seen at 3 months of age. At 45 days of age the experimental mouse (C) received whole body irradiation at a dosage of 600 rads, followed within 1 hour by an intravenous injection of 50 million spleen cells obtained from a normal littermate. Removal of calcified cartilage and bone has created zones of radiolucence in the subepiphyseal regions of the distal femur and proximal tibia, as indicated by arrows in (C). (D to F) Photomicrographs (× 30) of hematoxylin and eosin-stained sections of the proximal third of the tibia of normal control (D), osteopetrotic control (E), and experimental (F) mice at 1 month of age. At 10 days of age the experimental mouse (E) received an infusion of 23 million normal spleen cells within 2 hours after lethal irradiation. Replacement of the abnormal spongework of calcified cartilage and bone by hematopoietic tissue took place during the third week after administration of the transplant.

The mice used in this investigation were derived from heterozygous grey-lethal (*gl*/+) and microphthalmic (*mi*/+) breeders originally provided by the Jackson Laboratory. The microphthalmic mutant gene was crossed to the C57Bl/6J inbred strain. Both stocks have been inbred for over 20 generations. Nevertheless, reciprocal skin grafting tests indicated that neither stock was syngeneic. Therefore, consistently successful adoptive transfer experimentation required use of whole body irradiation at lethal dosages, which were determined to be 600 rads for osteopetrotic mice and 900 rads for normal mice. Radiation was administered from a cobalt-60 source to recipients within 2 hours before transplantation. The transplants were cell infusions prepared from the bone marrow (femora and tibiae) and spleen of normal littermates. Dispersal of the bone marrow cells was readily accomplished by repeated passage of the tissue through disposable hypodermic needles graded from 26 to 30 gauge. The spleen was minced with scissors before injection through the needles. Each sample was suspended in 100 to 200 μl of Hanks balanced salt solution and injected into the transverse facial vein of anesthetized recipients. As precautions against hemorrhage, anticoagulants were not used and the site of injection was tied off before removal of the needle. According to counts made on portions of each infusion, the number of nucleated cells delivered varied from 10 million to 25 million for bone marrow and 20 million to 50 million for spleen.

The influence of the myeloid cell infusions was examined in three different groups of mutants, which varied with respect to ages at onset and termination of the experiment. An infantile group included 11 grey-lethal (*gl*/*gl*) and 13 microphthalmic (*mi*/*mi*) mice, which were 10 to 12 days of age at onset and 30 to 42 days of age at termination; a juvenile group included 20 *mi*/*mi* mice, 20 to 25 days at onset and 110 to 115 days at termination; an adult group consisted of 18 *mi*/*mi* mice, 40 to 45 days at onset and 180 to 182 days at termination. In each series the number of mutants receiving bone marrow was nearly the same as the number given spleen cell infusions, and in most cases recipients were of the same sex as donors. Into each series the following controls were incorporated: (i) 12 normal mice that received normal bone marrow or spleen cells after whole body irradiation at 600 or 900 rads, (ii) 6 osteopetrotic mice that received cell infusions in the absence of radiation, (iii) 5

untreated normal mice, and (iv) 5 untreated osteopetrotic mice. Mice were housed in a constant-temperature room and fed a modified McCollum's I diet (*4*) both dry and wet.

During the observation period the body weight of each animal was measured twice a week; a tibial amputation was made at 2, 4, or 6 weeks after onset; and radiograms were made every 4 to 6 weeks. At autopsy several tissues were prepared for histologic examination, including bones (tibia, femur, and mandible) and lymphatic organs (spleen, lymph nodes, and thymus).

The normal bone marrow and spleen cell infusions restored capacity to resorb bone and calcified cartilage in all of the irradiated osteopetrotic recipients. The younger the recipient, the more rapid the rate of removal of skeletal matrix. Histologically, the earliest changes were detected 2 weeks after onset when an increase in mononuclear leukocytes was noted extravascularly along the vascular channels in the proximal third of the tibia. In the younger group of experimental mice, most of the excess bone and cartilage was removed from the long bones during the third and fourth weeks after onset. Figure 1F illustrates the proximal tibia of a grey-lethal mouse 30 days of age, which at 10 days of age received an infusion of normal spleen cells. The medullary cavity is fully expanded and filled with active hematopoietic tissue; the flare along the medial border is pronounced and the cortex is moderately well developed. These signs of recovery are appreciated more readily through comparison of the proximal tibia of the experimental mouse (Fig. 1F) with those of the untreated osteopetrotic (Fig. 1E) and normal (Fig. 1D) controls.

Mutants treated at the older ages required disproportionately more time to remove all of the excess matrix. Thus, the juvenile group of mutants required 6 to 9 weeks to make a complete recovery and the adult group required 12 to 24 weeks. The slower recovery rate of the older mutants may be accounted for by the increased extent and density of the spongiosa and the fact that fewer growth centers participated in the recovery. Radiologically, the first signs of recovery in the adult group of mutants were noted 6 weeks after onset, when well-defined zones of radiolucence appeared under the growth plates at the following sites: proximal humerus, iliac crest, distal femur, and proximal tibia. As the abnormal accumulations of calcified cartilage and bone were resorbed these zones of radiolucence extended along the length of the diaphysis until the expansion of the medullary cavity was complete. Detection of the subepiphyseal radiolucent zones in radiograms is facilitated by comparing the proximal tibia and distal femur of the experimental animal (Fig. 1C) with those of the untreated osteopetrotic (Fig. 1B) and normal (Fig. 1A) controls.

Examination of the skeletal and lymphoid tissues of the controls helped to establish that the mutants were immunologically competent and that neither radiologic damage nor graft-versus-host (GVH) disease played a direct role in the restoration of the capacity to resorb skeletal tissues. Osteopetrotic mice which received normal bone marrow or spleen cells in the absence of radiation never developed bone resorptive activity, which indicates that the donor cells had been destroyed. Irradiated normal mice receiving spleen or marrow cell infusions from normal littermates recovered uneventfully without manifesting the osteoporotic changes of GVH disease (*5*). In all of the control and experimental mice histologic examination of spleen and lymph nodes revealed the presence of well-developed thymus-dependent zones. Finally, unlike the mice with GVH disease, which are subject to progressive wasting, the experimental animals of the present study maintained about the same rate of body weight gain and skeletal growth after irradiation and transplantation as before.

The reversal of osteopetrosis in mice through bone marrow transplantation provides a new rationale for treatment of Albers-Schönberg marble bone disease in man.

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Spleen Cells Transmit Osteopetrosis in Mice

Abstract. *Osteopetrosis was induced in lethally irradiated, normal mice of grey-lethal and microphthalmic stocks by cell infusions prepared from the spleens of osteopetrotic littermates. Failure of skeletal remodeling became evident within a few weeks after transplantation as calcified cartilage and bone accumulated excessively in the active metaphyses of the long bones. The massive lesions produced were extensively infiltrated with abnormal osteoclasts.*

Bone resorption has been restored in osteopetrotic mice through parabiotic union of mutants and normal littermates and by the intravenous infusion of normal marrow and spleen cells into lethally irradiated mutants (*1*). Recovery from osteopetrosis was thought to be mediated by cells which had migrated via the bloodstream from their sites of origin in the hematopoietic tissues to the ossification centers, where they gave rise to a new population of competent osteoclasts or to cells that stimulate osteoclastic activity (*2*). The objective of the experiment reported here was to determine if the same mechanism would function in the reciprocal situation, with the osteopetrotic specimen serving as donor and the irradiated normal littermate as recipient in spleen cell transplantations. The osteopetrotic spleen cell infusions restored hematopoiesis but arrested skeletal remodeling, thus providing decisive evidence that bone resorption is under the direct control of the hematopoietic centers.

Weanling mice of grey-lethal (*gl/gl*) and microphthalmic (*mi/mi*) stock were distributed into four groups, 20 mice per group, identified as follows: (i) experimental splenic transplants, lethally irradiated normal mice that received spleen cell infusions from their osteopetrotic littermates; (ii) control splenic transplants, lethally irradiated normal mice that received spleen cell infusions from other normal littermates; (iii) untreated osteopetrotic controls; and (iv) untreated normal controls. Females and males were represented nearly equally in the above groups, and in most instances donors were of the same sex as recipients. About two-thirds of the mice in each group were obtained from microphthalmic stocks and one third from grey-lethal stocks.

Within 2 hours before infusion of cells, recipients were exposed to whole body irradiation at a dosage of 900 rads from a cobalt-60 source. Without anticoagulant, the thoroughly dispersed cells were administered via the transverse facial vein. As determined by a standard leukocyte counting procedure, the number of nucleated cells per injection ranged from 20 million to 40 million. For the first week after irradiation tetracycline and milk were given by stomach tube once a day. Thereafter the antibiotic was added to the drinking water and McCollum's diet I (*3*) was provided as a dry powder and wet mash. To monitor skeletal changes a tibia was obtained by hind limb amputation at 1 or 2 months af-