ULTRASTRUCTURAL FINDINGS OF BONE MARROW IN A CASE WITH MALIGNANT OSTEOPETROSIS FOLLOWING SUCCESSFUL ALLOGENEIC BONE MARROW TRANSPLANTATION*

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A nine-month-old female patient suffering from malignant osteopetrosis was evaluated by light and transmission electron microscopic study before and following allogeneic bone marrow transplantation (BMT). Bone marrow specimens were obtained from iliac crest biopsies. Before BMT, the bone marrow had an irregular appearance and was filled with bridging bony trabeculae devoid of cells. Following BMT, the marrow had an almost normal appearance with no myelofibrosis and a relatively regular distribution of hematopoietic cells. The osteocytes were visible in their lacunae in the bone matrix. Presence of bone resorbing and bone forming cell together demonstrated that the bone was beginning to gain its normal dynamic structure. These findings were in accordance with the clinical, laboratory and radiological data which showed the beneficial effect of the therapy. *Key words: malignant-infantile osteopetrosis, allogeneic bone marrow transplantation, ultrastructure.*

Osteopetrosis is an extremely rare disease characterized by skeletal sclerosis due to failure of osteoclast-mediated resorption and remodeling of bone. Two types of genetic transmission have been described[1,2]. The autosomal dominant pattern is relatively benign and diagnosed in adulthood. Most patients are asymptomatic and have normal life spans[3]. The classic form of infantile-malignant osteopetrosis is autosomal recessive (AR). Patients with the AR form of osteopetrosis have severe symptoms, including abnormal bone remodeling due to defective osteoclast function, deficient hematopoiesis and neurological impairments such as blindness and auditory nerve damage. Children affected by AR osteopetrosis have poor prognosis and usually die during the first decade of life[1,2,4].

Histologically, bone marrow spaces are narrow and usually contain fibrous tissue with very few elements of hematopoiesis. Bone trabeculae consist of a cartilage

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surrounded by an immature or woven bone[5,6]. The histology of the bone tissue, however, shows a considerable variation, with regard to number and activity of both the bone forming and resorbing cells[7,8].

A number of therapeutical approaches have been used in malignant osteopetrosis, including steroids, parathyroid hormones and cytokine therapies (M-CSF, IFN-ψ); however, response has been minimal and transient[9,10]. Because the cell origin of the osteoclast is the pluripotent hemapoietic stem cell, allogeneic bone marrow transplantation (BMT) has been applied successfully since 1911 (following animal experiments) for the correction of AR osteopetrosis[11-15].

In this report, we present ultrastructural findings of bone in a case with malignant osteopetrosis after a successful allogeneic bone marrow transplantation.

Material and Methods

Bone marrow specimens were obtained from the iliac crest of a nine-month-old female patient suffering from malignant osteopetrosis. Biopsies were taken before and three months after allogeneic BMT. Tissue specimens were fixed in 2.5 percent glutaraldehyde in Sorensons' phosphate buffer, and decalcified in sodium 0.1 M EDTA (EDTA disodium salt, Sigma) solution. After washing in PBS, they were postfixed in one percent osmium tetroxide in PBS at 4 °C for one hour. Specimens were then dehydrated in a graded series of ethanol to absolute ethanol in preparation for embedding in araldite Cy 212 (agar). Semi-thin sections were stained with methylene blue azure II, and thin sections with uranyl acetate and lead citrate, before being examined and photographed.

Results

Semi-thin sections were examined to study the bone matrix, the marrow cavity, the osteoblasts and the osteoclasts in terms of size, number, nucleation and relationship to the persistent matrices.

The light microscopic observations on the semi-thin sections before BMT revealed irregularly shaped bone trabeculae consisting of large cartilage cores surrounded by an immature (woven) bone material occupying most of the sections so that intervening marrow spaces were very narrow. Hematopoietic elements were nearly absent in the marrow cavities. Bone matrix was irregular in density. Neither bone forming (osteblast) nor bone resorbing (osteoclast) cells were seen in the semi-thin sections, indicating that bone formation and resorption were affected. Furthermore, there was no clear evidence of main bone cells (osteocytes) embedded in the bone matrix (Fig. 1).

Three months after BMT, semi-thin sections revealedmany large osteoclasts located within the resorption cavities they produce. Some osteocytes were embedded in the matrix, whereas highly active osteoblasts are usually found

in the bone surfaces. Osteoclast number, size and nucleation varied from normal to increased levels. The marrow tended to have an almost normal appearance with no myelofibrosis and a relatively regular distribution of hematopoietic cells. The presence of bone resorbing and bone forming cells together demonstrated that the bone was beginning to gain its normal dynamic structure. Collagen synthesized by the osteoblasts was apparent. In the semi-thin and thin sections, osteoblasts appeared to be highly active (Figs. 2, 3, 4).

Fig. 1: Light micrograph before bone marrow transplantation (BMT) showing large cartilage cores (arrow) and woven bone (double arrow), (x 10, methylene blue azure II).

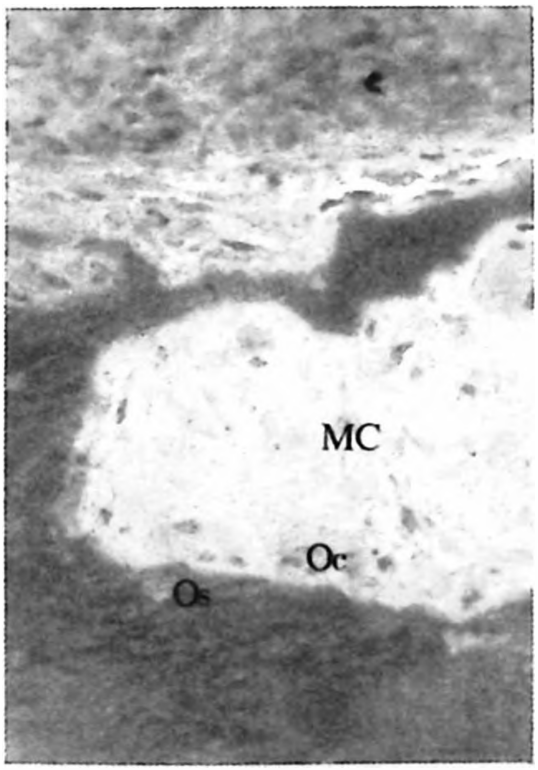

Fig. 2: Light microscopic appearance of bone and bone marrow after bone marrow transplantation (BMT). Osteocytes (Os), osteoclasts (Oc) and the cellular elements in the marrow cavity (MC) are observed, (x 40, methylene blue azure II).

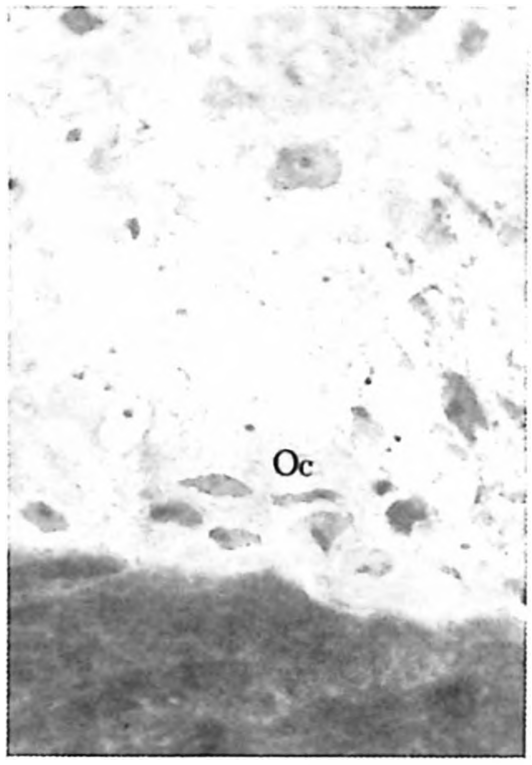

Fig. 3: A large multinucleated osteoclast (Oc) on the surface of a bone trabecula, (x 100, methylene blue azure II).

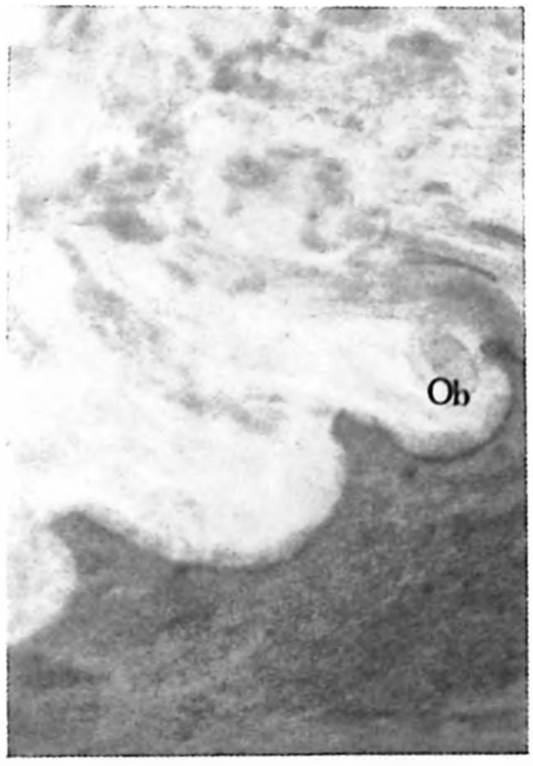

Fig. 4: Resorption cavities in he margin of the bone trabeculae, and an osteoblast (Ob) forming the collagenous matrix, (x 40, methylene blue azure II).

Electron microscopic observations on the thin sections three months after BMT revealed that the osteoblasts were numerous and active (synthesizing the collagen component of the matrix). They had a normal appearance, with a well developed rough endoplasmic reticulum, euchromatic nucleus, and prominent nucleolus. The collagen fibers were normal in terms of periodicity, size, and shape (Fig. 5). The osteocytes in their lacunae had a normal mature and inactive appearance. Osteoclasts were infrequently observed. They were large, rich in cytoplasmic vesicles and vacuoles, and had active and prominent irregularly shaped nucleoli (Fig. 6). The ruffled border and the clear zone complexes, however, were not evident.

Fig. 5: Electron micrograph showing the many active bone forming cells (osteoblast). Cytoplasm contains many rough endoplasmic reticulum cisternae. Section of collagen matrix is seen arround the cells. Dark areas (arrow) represent the onset of calcification in the osteoid matrix, (x 13,500, uranyl acetate-lead citrate).

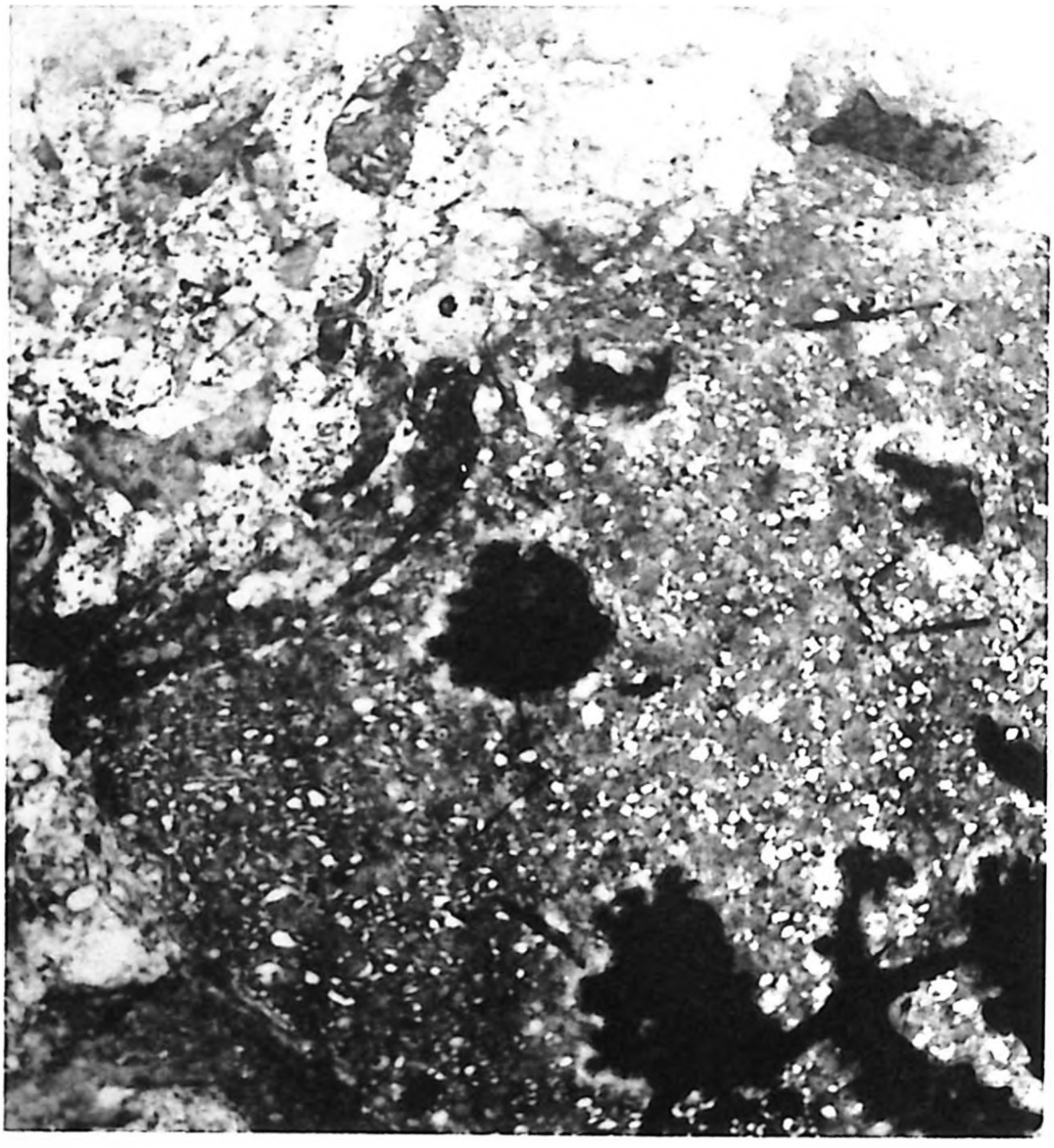

Fig. 6: Electron micrograph of an osteoclast after bone marrow transplantation (BMT). The cytoplasm is filled with vacuoles, (x 25,500, uranyl acetate-lead citrate).

Discussion

Our case had the symptomatology of malignant osteopetrosis including optic atrophy, nistagmus, bone fractures, hepatosplenomegaly, anemia, and thrombocytopenia. The patient had a history of sibling death with the same disorder. We performed bone marrow transplantation from her HLA-matched healthy sibling, and the patient showed clinical and laboratory improvement and was cured. Before BMT, the osteopetrotic bone consisted of an amorphous organic material with an acellular appearance. Following BMT, a new and healthy bone formation was beginning. Active bone formation together with bone resorption demonstrated physiological remodeling. The specific plasticity of bone is provided by a sufficient number of osteocytes. In thin sections the osteoclasts

did not show ruffled border-clear zone complexes, but they were present and active in the resorption cavities. The bone marrow appeared relatively normal compared to the period prior to transplantation.

We still do not know definitively whether the failure of effective bone resorption is due to intrinsic osteoclast abnormalities or to extrinsic factors such as matrix abnormalities. The extrinsic factors may make osteoclasts ineffective; however, it has been suggested that a strong reduction in the number of osteoblasts in an osteopetrotic patient may have a negative influence on the functioning of the osteoclasts and even on hematopoiesis[6]. On the other hand, it is also conceivable that the opposite occurs: that an osteoclastic abnormality influences osteoblastic activity leading to an abnormal bone deposition (woven bone). Our electron microscopic observations after BMT showed that osteoblastic activity was very prominent, indicating that both apparently normal endochondral bone formation and bone resorption had already begun.

Ultrastructural studies document the heterogeneity of the bone marrow in AR osteopetrosis[6,7]. Although diminished, the absent ruffled border-clear zone complexes of osteoclasts can be observed in the majority of osteopetrotic biopsies; extensive complexes are even noted in some cases[7]. The profiles of normal controls indicate that not all osteoclast membranes adjacent to bone and cartilage are thrown into a ruffled border conformation. Thus, it is not correct to note an area of non-ruffling by ultrastructure and assume that all the cells are inactive or, in reverse, to indicate that all areas of ruffling imply effective activity[7]. In this case report, active appearance of the osteoclasts within the resorption cavities in the presence of a healthy bone matrix and osteoblastic cells were found in association with an improvement of clinical, laboratory and radiological parameters. The patient engrafted rapidly and the new bone formation was of the same density as the original bone.

Careful morphological bone biopsies after BMT in combination with more delicate cell biological studies, related to the function of bone forming and resorbing cells, are needed to clarify the pathogenesis and treatment of the disease.

REFERENCES

1. Johnston CC, Lavy N, Lord T, Vellios F, Merritt D, Deiss WP. Osteopetrosis: a clinical, genetic, metabolic, and morphologic study of the dominantly inherited benign form. Medicine 1968; 47: 149-167.
2. Cortes RL, Calvo EQ, Chaverri CC. Osteopetrosis in children: a report of 26 cases. J Pediatr 1977; 91: 43-47.
3. Walpole IR, Nicoll A, Goldblatt J. Autosomal dominant osteopetrosis type II with "malignant" presentation:; further support for heterogeneity? Clin Genet 1990; 38: 257-263.
4. Gerritsen EJ, Vossen JM, van Loo IH, et al. Autosomal recessive osteopetrosis: variability of findings at diagnosis and during the natural course. Pediatrics 1994; 93: 247-253.

5. Coe FL, Favus MJ. Disorders of Bone and Mineral Metabolism. New York: Raven Press; 1992: 988-995.
6. Shapiro F, Key LL, Anast C. Variable osteoclast appearance in human infantile osteopetrosis. Calcif Tissue Int 1988; 43: 67-76.
7. Helfrich MH, Aronson DC, Everts V, et al. Morphologic features of bone in human osteopetrosis. Bone 1991, 12: 411-419.
8. Elster AD, Theros EG, Key LL, Stanton C. Autosomal recessive osteopetrosis: bone marrow imaging. Radiology 1992; 182: 507-514.
9. Key LL, Rodriguiz RM, Wang WC. Cytokines and bone resorption in osteopetrosis. Int J Pediatr Hematol 1995; 2: 143-149.
10. Sieff CA Chessels JM, Levinsky RJ, Pritchard J, Rogers DW. Allogeneic bone-marrow transplantation in infantile malignant osteopetrosis. Lancet 1983; 26: 437-411.
11. Gerritsen EJ, Vossen JM, Fasth A, et al. Bone marrow transplantation for autosomal recessive osteopetrosis. J Pediatr 1994; 125: 896-902.
12. Key LL JR, Rodriguiz RM, Willi SM, et al. Long-term treatment of osteopetrosis with recombinant human interferon gama. N Engl J Med 1995; 332: 1594-1599.
13. Solh H, Da Cunha AM, Giri N, et al. A. Bone marrow transplantation for infantile malignant osteopetrosis. J Pediatr Hematol Oncol 1995; 17: 350-355.
14. Taylor GM, Dearden SP, Will AM, et al. Infantile osteopetrosis: bone marrow transplantation from a cousin donor. Arch Dis Child 1995; 73: 453-455.
15. Monaghan BA, Kaplan FS, August CS, Fallon MD, Flannery DB. Transient infantile osteopetrosis. J Pediatr 1991; 118: 252-256.