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HYPERCALCEMIA FOLLOWING UMBILICAL CORD BLOOD TRANSPLANTATION TO CORRECT OSTEOPETROSIS ASSOCIATED WITH THE *NEMO* MUTATION

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Abstract X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) is a congenital developmental and immunologic disorder, which often causes osteopetrosis. These individuals are susceptible to infection with various microorganisms, and most patients die of infection before reaching maturity. Hematopoietic stem cell transplantation (HSCT) is considered the only cure for this disorder. When HSCT is performed in patients with osteopetrosis, on the other hand, transient hypercalcemia often occurs in these patients. We report a XL-EDA-ID patient with osteopetrosis who experienced hypercalcemia and resolution of bone changes after cord blood transplantation.

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X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) is a congenital developmental and immunologic disorder caused by hypomorphic mutations in the nuclear factor-kappa B essential modulator (*NEMO*) gene on the X chromosome.[1-3] Affected children, most often boys have sparse hair, eccrine sweat gland dysgenesis, characteristic facies, and few teeth, with those present being conical in shape; patients may also have severe intestinal problems and osteopetrosis.[4] These individuals are susceptible to infection with various microorganisms, suffer lethal infections at infancy, and most patients die of infection before reaching maturity.

Malignant infantile osteopetrosis, caused by osteoclast dysfunction, is another lethal disorder that leads to bone marrow failure for which hematopoietic stem cell transplantation (HSCT) is considered the only cure.[5] In addition, autosomal recessive osteopetrosis is also caused by osteoclast dysfunction. HSCT has been successfully applied to the patients.[6] When these treatments are successful, patients experience transient hypercalcemia and demonstrate improvement in bone sclerosis. Here, we report a XL-EDA-ID patient with osteopetrosis who experienced hypercalcemia and resolution of bone changes after cord blood transplantation.

A 2-month-old boy was referred to our hospital for anemia and frequent episodes of sepsis. His mother, as well as his maternal grandmother and her sister, carried the diagnosis of familial incontinentia pigmenti (IP). The patient's maternal uncle died from unknown causes at the age of 1 month. The patient had mild facial dysmorphy, with relative frontal bossing and a depressed nasal bridge, and anhidrotic skin.

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Laboratory examination confirmed anemia and showed hypogammaglobulinemia, low natural killer (NK) cell activity, and high numbers of B cells. A skin biopsy revealed the absence of eccrine sweat glands. Radiography showed expansion of the metaphyses and obstruction of the medullary cavity in the long bones; these findings were consistent with osteopetrosis. Although anemia was noted, there were no signs of extramedullary hematopoiesis such as leukopenia, thrombocytopenia, or hepatosplenomegaly, and there were no symptoms of cranial nerve compression.

NEMO is the regulatory subunit of the inhibitor of kappa B kinase complex, which phosphorylates and degrades NF-κB inhibitor a, causing activation of NF-κB. Following the discovery that amorphic *NEMO* mutations cause IP, hypomorphic mutations in *NEMO* have been identified in patients with XL-EDA-ID.[1-3] In our patient, nucleic acid extracted from peripheral blood mononuclear cells (PBMC) was analyzed for the *NEMO* mutation. A C insertion was identified at base 1167 in exon 10, resulting in a predicted frameshift at codon 390 and early termination.[4] He was diagnosed with XL-EDA-ID and started on biweekly treatment with intravenous gamma globulin, however he had frequent episodes of enterocolitis and *Enterobacter aerogenes* sepsis. At 2.5 years of age, he suffered severe pyloric obstruction caused by gastric ulcers, following a bout of enterocolitis. Oral feeding was stopped and the patient began receiving total parenteral nutrition.

Because he did not have a human leukocyte antigen-matched donor, related or unrelated, an unrelated umbilical cord blood transplantation (UCBT) was performed at 3 years of age, using a male donor (Figure 1).[4] The pretransplant conditioning consisted of fludarabine, 30 mg/m^2 on days -7 to -3 (total dose, 150 mg/m^2), melpharan, 70 mg/m^2 on days -6 to -5 (total dose, 140 mg/m^2), and rabbit anti-T-lymphocyte globulin, 2.5 mg/kg on days -5 to -1 (total dose, 12.5 mg/kg). The

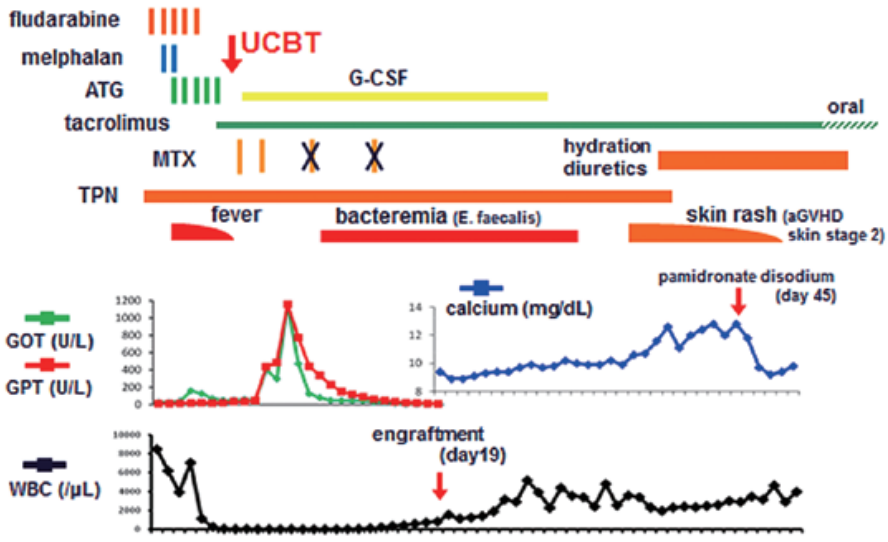

Figure 1. Clinical course of umbilical cord blood transplantation
UCBT: umbilical cord blood transplantation
ATG: rabbit anti-T-lymphocyte globulin
MTX: methotrexate
TPN: total parenteral nutrition

patient received a transplantation of 4.6×10^7 total nucleated cells/kg. The recipient and donor were 1 antigen mismatched by serology and 4 loci mismatched by DNA typing. Tacrolimus and short-term methotrexate were used for graft-versus host disease (GVHD) prophylaxis. Grade I acute GVHD (skin stage 2) did occur on day +35, but resolved without steroid therapy. *NEMO* protein expression normalized in all PBMC lineages, and full donor chimerism was achieved in all cell lineages on day +105 (data not shown).

Prior to the UCBT, the patient's serum calcium levels were normal; these levels increased progressively from day +36 and reached their maximum on day +43 (13.0 mg/dL), despite initial treatment including diuresis with both saline and furosemide. Because this hypercalcemia persisted over the following 2 days, 0.5 mg/kg pamidronate disodium was administered on day +45. The serum calcium levels normalized within 2 days. The patient never experienced symptoms of hypercalcemia and no adverse events were observed with the use of pamidronate disodium. Hypercalcemia occurred twice more, but spontaneously improved without treatment. The patient was discharged on day +114 with a perfect performance status. One year after transplantation, we noted correction of his osteopetrosis and 5 years after transplantation, radiography revealed further improvement of the osteopetrosis in his lower limbs. Both his immunodeficiency and osteopetrosis were successfully corrected by UCBT.

The differentiation of osteoclasts is dependent on a receptor activator of NF-κB ligand[7] whose signaling pathway is probably impaired in patients with *NEMO* disorders.[2] Because osteoclasts are derived from hematopoietic stem cells,[8, 9] we believe that the patient's osteoclasts were replaced by those derived from the donor, and that their function was reconstituted by the UCBT.[5, 6]

Hypercacemia is common in patients with osteopetrosis after hematopoietic stem cell transplantation.[10] This suggests a microenvironment renewal by donor-derived osteoclasts that results in an increased release of calcium, previously stored in the osteopetrotic bones. Children older than 2 years of age have a higher risk of hypercalcemia than infants, because of their relatively large bone mass.

In summary, we noted hypercalcemia and correction of osteopetrosis in a patient with XL-EDA-ID after UCBT, both effects presumably resulting from reconstitution of osteoclast function. Our experience shows that correction of immunodeficiency and osteopetrosis associated with the *NEMO* mutation is possible with UCBT.

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