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Secondary G-CSF Mobilized Blood Stem Cell Transplantation without Preconditioning in a Patient with Gaucher Disease:

Report of a New Approach which Resulted in Complete Reversal of Severe Skeletal Involvement

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Gaucher disease has been treated by allogeneic bone marrow transplantation (BMT), however, severe bone involvement that is probably the most disabling aspect of this disease is difficult to reverse. Other problem of BMT is the use of intensive preconditioning that adversely affects growth and development of the patients.

In this study, a patient with type I Gaucher disease was treated by allogeneic BMT from HLA-matched sibling donor. However, the treatment resulted in late graft failure and the patient developed severe bone involvement. Fifty months after the first BMT, the patient was treated by allogeneic peripheral blood stem cell (PBSC) transplantation without preconditioning. Recombinant human granulocyte-colony stimulating factor (rhG-CSF) was used to mobilize PBSC. Cyclosporine A (CyA) was administered for the prophylaxis of graft-versus-host disease (GVHD). Full donor-derived hematopoiesis was obtained, and clinical symptoms including severe bone involvement improved completely with increased glucocerebrosidase activity.

It was shown that an engraftment could be obtained without intensive preconditioning when a recipient receives an rhG-CSF-mobilized PBSCs infusion as a secondary transplant. Another important finding of this study is the complete reversal of severe bone involvement by the supply of abundant glucocerebrosidase from high proliferating PBSC graft.

Key words : Gaucher disease, allogeneic PBSCT, competitive repopulation, skeletal involvement

INTRODUCTION

Allogeneic bone marrow transplantation (BMT) has been used to treat Gaucher disease, and remarkable improvement of performance status was reported [1-3]. However, the efficacy of BMT on Gaucher disease is limited to soft tissue in most cases, and few patients have been cured of the severe bone damages. Another problem with using BMT to treat Gaucher disease is the intensive preconditioning regimen necessary to obtain durable engraftment [4, 5]. Most of the preconditioning regimens consist of irradiation or high dose busulfan (Bu) and cyclophosphamide (Cy), and these agents affect the growth and development of the patients negatively [6, 7].

The patient presented here received recombinant human granulocyte colony stimulating factor (rhG-CSF) mobilized peripheral blood stem cell transplantation (PBSCT) after a preceding BMT had resulted in late graft failure. Durable engraftment without preconditioning was accomplished successfully. The patient's multiple bone

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fractures were cured completely after the secondary PBSCT, and clinical performance status has dramatically improved.

CASE REPORT

A two-year-old female with Gaucher disease was referred to the hospital to receive allogeneic BMT from her HLA-identical, ABO-minor mismatched (patient was A, donor was O) sister in January 1989. A splenectomy was performed on the patient in February 1989 to prevent graft rejection [3]. The patient received preconditioning which consisted of 6 Gy of thracoabdominal irradiation (TAI; 3 fractioned) and 200 mg/kg of Cy, and both short term methotrexate (MTX) and cyclosporine A (CyA) were given as prophylaxis for graft-versus-host disease (GVHD). Because the patient's myelosuppression was slow, 50 mg/kg of antilymphocyte globulin (ALG, Lymphosa, Berne, Switzerland) was administered on the day before marrow infusion. A total of 5.75×10^8/kg of bone marrow nucleated cells were infused though a central venous catheter line on April 13, 1989. Her hematopoietic recovery was prompt, and neither acute nor chronic GVHD were observed. However, restriction fragment length polymorphism (RFLP), using genomic DNA of glucocerebrosidase as a probe [7], showed partial chimerism, and donor type RBCs increased only transiently. The activity of glucocerebrosidase was elevated about 40% of normal control and 60% of donor's level, and clinical improvement (reduced liver size, increased muscle power) was obtained. The patient was followed in the outpatient clinic in the hospital for three years. During that time, two episodes of right and left femoral bone fracture developed and the patient was admitted to the local hospital. After two episodes of bone fractures, the patient was unable to walk.

In June 1993, the patient was readmitted to the hospital. Physical examination revealed systemic atrophy of skeletal muscles, and the patient could not raise her arms above her shoulders. On X-P examination, both bilateral Humeri and Femur were fractured, and both shoulder and hip joints were markedly deformed. The patient's blood counts were: white cells, 13,900/μl; hemoglobin, 11 g/dl; and platelets, 463,000/μl. A decision was made to perform rG-CSF mobilized PBSCT using the same donor without preconditioning after informed consent was obtained from the patient's family members. The donor received 2 μg/kg of rhG-CSF subcutaneously from June 28 through July 2 and leukaphresis was performed on July 2 and 3. A total of 9.0×10^8/kg of nucleated cells was harvested and infused. The dose of CFU-GM was 9.5×10^4/kg. The patient received 4 mg/kg of CyA orally for the prophylaxis of GVHD. Acute GVHD was not observed and the patient was discharged 31 days after the first PBSCT.

Seven weeks after the secondary PBSCT, glucocerebrosidase activity increased to 80% of normal level, and RFLP analysis showed dominancy of the donor type. Two months after the secondary PBSCT, pancytopenia (white cells, 2,800/μl; hemoglobin, 5.1 g/dl; and platelets, 13,000/μl) developed and the patient was admitted to the hospital on September 14, 1993.

On physical examination, marked anemia and petechiae were observed, however her other physical findings were the same as those found her second admission. Both prednisolone and danazol were administered to treat pancytopenia, but were ineffective. Thereafter, the patient was treated with both rhG-CSF and erythropoietin (Epo). Twenty-five days after the cytokine treatment began, her white blood cell began to increase, and both rhG-CSF and Epo were stopped. The patient was discharged from the hospital on March 1, 1994 with the following hematological finding: white cell count, 4,800/μl; hemoglobin, 12.1 g/dl; platelets, 48,000/μl. Her red blood cell type had changed from A (+) to O (+), a feature that did not occur after the first BMT.

During the last admission, the patient's physical findings improved, including dental development, reversal of skeletal muscle atrophy, and mobility of shoulder's joints, the patient was able to stand by herself. Ten-years after secondary PBSCT, the patient has been enjoying normal school life with persistent donor-derived hematopoiesis.

ABO blood type during two stem cell transplantations (Fig. 1): After the first BMT, O (+) RBC increased mere than 50% until two months posttransplantation, however became decreasing less than 10% 3 months posttransplantation and finally reached to

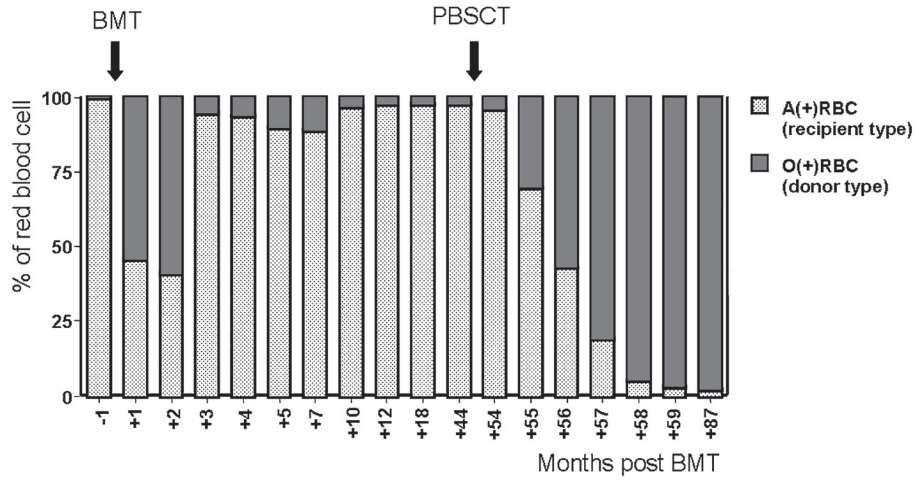

Fig. 1 Percentage of donor type RBC after primary BMT and secondary PBSCT.
Donor type RBC increased only transiently up to 15%, then decreased to background level after primary BMT. However, donor type RBC increased more than 97% after PBSCT and durable engraftment was achieved.

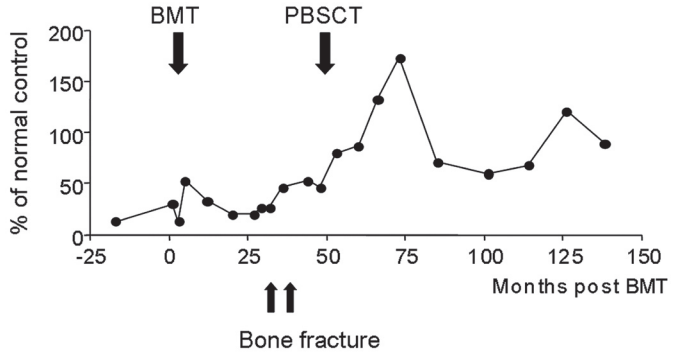

Fig. 2 Glucocerebrosidase activity after primary BMT and secondary PBSCT.
Glucocerebrosidase activity increased only transiently up to 47%, and then decreased to pretransplant level. However, rapid increase of Glucocerebrosidase activity was observed after secondary PBSCT.

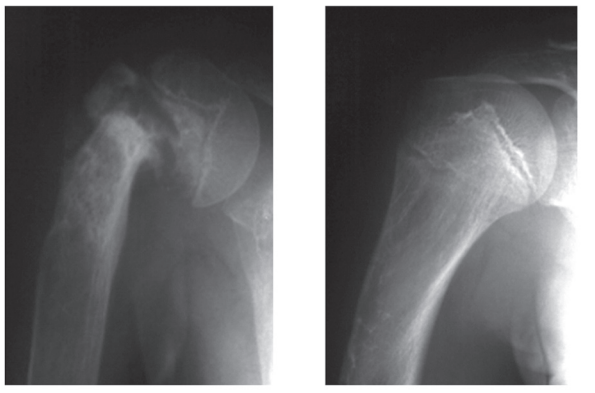

Fig. 3 Bone X-P findings of right shoulder joint.
Before the secondary PBSCT, diffuse osteopenia, osteonecrosis of the proximal Humerus were observed. Caput Humeri was dislocated from shoulder's joint. These findings were completely corrected 42 months after secondary PBSCT.

background level. After the PBSCT, O (+) RBC increased again to more than 97% by 9 months posttransplantation.

Glucocerebrosidase activity during two stem cell transplantations (Fig. 2): Glucocerebrosidase activities in the patient's lymphocytes were measured by the standard technique [9, 10] and compared with healthy controls. Glucocerebrosidase activity increased transiently 5 months after BMT and then varied within 13 to 25% of normal control. One month after PBSCT, glucocerebrosidase activity began to increase and reached to normal levels 13 months posttransplantation.

Bone X-P findings of right shoulder joint (Fig. 3): Before the secondary PBSCT, diffuse osteopenia, osteonecrosis of the proximal Humerus were observed. Caput Humeri was dislocated from shoulder's joint. All severe skeletal manifestations improved markedly as early as 1 year and reversed completely 42 months post PBSCT.

DISCUSSION

The efficacy of allogeneic BMT in the treatment of lysosomal storage disease depends on the amount of the primary deficient enzyme contained in donor derived blood components. Because the aim of allogeneic BMT in patients with congenital metabolic diseases is to provide sufficient amounts of the deficient enzyme supplied by hematopoietic cells, highly donor-dominant mixed chimeric status is sufficient for these patients. Because it is not necessary to eradicate the malignant cells, engraftment of donor derived hematopoietic cells without the use of intensive preconditioning is the ideal BMT for metabolic diseases even small recipient-derived hematopoiesis may persist.

High dose BuCy has been frequently used as the preconditioning for allogeneic BMT in patients with congenital metabolic disease [5]. However, regimen related toxicities (RRT) of BuCy including hepatic venoocclusive disease (VOD), hemorrhagic cystitis, interstitial pneumonitis and cataract have been reported. These RRT clearly affect the quality of the patient's life adversely after allogeneic BMT. The problem is the same when a patient is preconditioned by TAI and CY, although the frequencies and severities of RRTs are somewhat different.

It has been believed that preconditioning before donor-marrow infusion is essential to achieve successful engraftment [4, 5]. Preconditioning exerts both immunosuppressive and myelosuppressive effects on recipient marrow and creates a niche for the stem cell graft. However, it is possible to repopulate a recipient with a high dose of donor marrow cells without eradicating recipient stem cells in murine model [11]; a process usually required to overcome competitive hematopoiesis. For this procedure to be successful, two conditions must exist: 1) there must be no competitive disadvantage for donor cells; and 2) donor cells possess highly proliferating potential.

The patient presented here received allogeneic BMT from an HLA-matched sibling first and though this did not result in durable engraftment, the patient had small population of donor-derived hematopoiesis for 6 months following the BMT from the result of ABO-blood typing. A tolerance may be established between donor cells and recipient cells before secondary PBSCT because of long-term mixed chimerism. This fact may help donor PBSCs to proliferate in marrow space. Another major factor for the success of engraftment in this patient is the highly proliferating potential of rhG-CSF mobilized PBSCs. It has been reported that the patients received allogeneic PBSCT show rapid recovery of hematopoiesis compared to the patients who received allogeneic marrow grafts. The stem cells contained in rhG-CSF mobilized peripheral blood are already primed and can overcome the recipient marrow cells in the tempo of proliferation.

More than 80% of patients with Gaucher disease have some degree of skeletal involvement and more than half of these will experience serious complications including osteopenia, osteosclerosis, osteonecrosis, with collapse of major joints and pathologic fractures that lead to severe disability [12]. Allogeneic BMT has the effect of enzyme replacement therapy and improves both soft tissue and hematologic involvements [1-3]. However, the effect of BMT on severe skeletal damage is not obvious. Recently, macrophage-targeted glucocerebrosidase has been used to treat patients with Gaucher disease, and both hematologic and visceral responses have been obtained similar to that found in patients who received BMT [13, 14]. With administration of large doses of macrophage-targeted glucocerebrosidase,

some patients showed improved skeletal involvement after more than three years of therapy [15]. The patient reported in this paper showed improvement of severe skeletal damage that was evident on X-ray as early as one year after PBSCT, and showed the complete reversal of the dissociation of fractured bones three years after PBSCT. Previous report on long-term follow-up after BMT for Gaucher disease showed that the skeletal involvement had not improved in 3 patients or had deteriorated in 1 patient [16]. Incidence of mixed chimerism that leads to decreased glucocerebrosidase supply is less common in PBSCT than BMT and hematopoietic recovery is also faster in PBSCT than BMT [17]. PBSCT for metabolic diseases may be superior to BMT from the point of enzyme replacement therapy.

It is often observed that transient overshooting of primary deficient enzyme activity during a certain period after allogeneic BMT in recipients of congenital metabolic diseases. The mechanism of this overshooting is not exactly known, however, this overshooting of enzyme activity may contribute to rapid clinical improvement.

Allogeneic BMT may have a limited role in the treatment of Gaucher disease after the development of macrophage-targeted glucocerebrosidase therapy. However, engraftment without the use of preconditioning, and rapid reversal of severe skeletal damage, both observed in this patient constitute new strategy for the treatment of Gaucher disease. Future studies must focus on the development of new methods of allogeneic PBSCT with the use of tolerance-inducing treatment in place of intensive preconditioning. For example, subcutaneous injection of irradiated-donor lymphocytes preceding allogeneic stem cell transplantation may be effective to induce immune tolerance between recipient and donor and make it possible to achieve durable engraftment of donor-derived hematopoietic stem cells without intensive preconditioning. At least, secondary G-CSF mobilized PBSCT from the same donor without preconditioning should be tried as a first-line treatment of late graft failure.

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