조혈모세포이식 후 후기생착 실패를 보인 중증 재생불량빈혈 환자에게서 선택적 CD34$^+$ 세포의 성공적 주입

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Successful Selective CD34$^+$ Cell Infusion after Late Graft Failure of Hematopoietic Stem Cell Transplantation in Two Cases of Severe Aplastic Anemia

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Matched sibling bone marrow transplantation (BMT) in severe aplastic anemia (SAA) has been known as the treatment of choice in children and young adults. To overcome graft failure, second stem cell transplantation showed good results in previous studies. Here we report two cases of aplastic anemia patients with late graft failure and resulted in successful complete recovery after selective CD34$^+$ cell boost infusion. The patients previously underwent allogeneic BMT from HLA-matched sibling donors and the engraftment was achieved although their CBC started to decrease respectively 3 months and 11 months after transplantation. Both patients received selective CD34$^+$ cell infusion without additional conditioning therapy. Their CBC showed significant improvement and they are doing well without transfusion or complications. From this study we suggest that selected CD34$^+$ cell boost treatment can be a promising curative treatment for late graft failure after matched sibling BMT in SAA patients.

Key Words: Aplastic anemia, Late graft rejection, Selective CD34$^+$ cell infusion

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Introduction

HLA-matched sibling bone marrow transplantation (BMT) in severe aplastic anemia (SAA) has been known as the treatment of choice in children and young adults [1], although graft failure occurs in 5-25% of patients after transplantation [2]. Unlike primary graft failure which means no engraftment of donor cells, secondary graft failure is associated with loss of donor chimerism after engraftment [3] showing marrow hypoplasia with a requirement of frequent transfusions [4]. To overcome graft failure which leads to increased mortality and morbidity, second stem cell transplantation is suggested in several previous studies [3-5]. Cells of the same donor or other allogeneic donor have been used for the boost treatment, and moreover selective CD34$^+$ cells are proposed to be a successful source of re-infusion in graft failure [6].

We report our experience of the selective CD34$^+$ cell boost treatment in two aplastic anemia patients who had

late graft failure after matched sibling BMT.

Case Reports

1) Case 1

An 11-year-old girl underwent allogeneic BMT from her HLA (8/8) and ABO matched sister 11 months after the diagnosis of SAA. Fanconi anemia and other myelodysplastic syndromes were excluded. The conditioning therapy included cyclophosphamide (50 mg/kg) for 4 days and anti-thymocyte globulin (ATG) (2.5 mg/kg) for 3 days. Infused $CD34^+$ cell dose was 1.4×10^6/kg of recipient. The engraftment, which is defined as the first day of three consecutive days of more than 0.5×10^9/L of absolute neutrophil count (ANC), was achieved on day +22 and on day +25 bone marrow (BM) biopsy showed hypocellular marrow with complete donor chimerism. The patient received granulocyte colony-stimulating factor from day +3 and cyclosporine and methotrexate were used for GVHD prophylasix. She had no symptoms and signs of acute graft versus host disease (GVHD). On post-transplant day +88, her CBC showed decreased result of WBC 1,880/μL, hemoglobin 9.1 g/dL, and platelets 2.9×10^3/μL. Day +99 BM biopsy showed hypocellular marrow (cellularity of less than 5%) in complete donor chimerism without change. Since then, her CBC steadily decreased, she started to require RBC and platelet transfusions by 2 years after transplantation. Four years after BMT, her sister's peripheral blood stem cells were collected for the treatment of late rejection. We used CliniMACS sorting device (Miltenyi Biotec) to select $CD34^+$ cells at post-transplant 52.4 months, $CD34^+$ cells of 1.83×10^6/kg were infused as boost therapy. No additional conditioning drugs or GVHD prophylaxis were used and no complications occurred during and after the infusion. Until now, by 2 years after the boost treatment, the patient is being well with stable engraftment.

2) Case 2

A 15-year-old girl who had a history of idiopathic aplastic anemia and sibling BMT visited our hospital under the diagnosis of late graft rejection. She was diagnosed as aplastic anemia at the age of 13, and received BMT from HLA matched (8/8) but ABO and Rh mismatched brother, in another hospital. Conditioning regimen consists of cyclophosphamide (50 mg/kg) for 4 days, ATG (2.5 mg/kg) for 3 days and procarbazine (12.5 mg/kg) for 3 days was undergone. The $CD34^+$ cells of 0.18×10^6/kg were infused and on day +16, ANC increased up to 0.5×10^9/L. Although the engraftment was successful the graft rejection was suspected after 11 months of the BMT. Laboratory results at the time of her first visit to our clinic were as follows: WBC 2,770/μL, hemoglobin 6.3 g/dL, and platelets 3.1×10^3/μL. She received regular check-ups in our outpatient clinic. During follow-up, her pancytopenia progressed and BM biopsy showed hypocellular marrow (cellularity 10-20%) with complete donor chimerism. Five years after BMT, a dose of 8.38×10^6 selective $CD34^+$ cells per recipient body weight were administered without conditioning and GVHD prophylasix. During the 1 year follow-up, she has shown recovery of cell counts with no complications or GVHD.

Discussion

In this report, patients of SAA showed successful engraftment after sibling BMT with full donor chimerism although sustained stabilized hematopoiesis was not successfully achieved during follow-up. We have chosen $CD34^+$ cells boost treatment for rescue therapy, and two weeks after the boost infusion, patients' CBC showed complete recovery. They have been being followed up in the outpatient clinic without transfusion for 28 months (case 1) and 16 months (case 2). Their CBC results after the boost treatment show significant increase compared to the results before their boost treatment. In case 1, median WBC (2,490±959/μL vs. 5,149±1,072/μL, $P<0.001$), median hemoglobin (6.6±1.4 g/dL vs. 10.5±1.6 g/dL, $P<0.001$), and median platelets ($3.52\pm1.27\times10^3$/μL vs. $8.28\pm3.57\times10^3$/μL) all increased. In case 2, median WBC (3,113±474/μL vs. 3,636±555/μL, $P=0.01$), median hemoglobin (8.5±1.4 g/dL vs. 11.5±2.0 g/dL, $P<0.001$), median platelets ($4.05\pm0.96\times10^3$/μL vs. $9.44\pm2.83\times10^3$/μL) showed similar results.

Graft rejection is one of the most life threatening complications of BMT. Low stem cell dose, patients receiving transplantation from an HLA-mismatched, from an un-

related donor, T cell-depleted graft, viral or fungal infection after transplantation and GVHD are known factors that increase incidence of graft failure [5,6]. To prevent graft failure, large doses of stem cell infusion, matched donor transplantation, lesser transfusion to lower sensitization have been suggested although the cause of graft failure is still not well known and it is difficult to predict graft failure [4,7].

To overcome this condition, boost treatment or second hematopoietic stem cell transplantation is being performed. One of the option is reinfusion of stem cells from a same donor with or without preparative conditioning treatment or from an alternative donor. Nevertheless, prolonged pancytopenia and additional conditioning regimens increase complications such as infections and GVHD resulting in poor prognosis of graft failure [3-5].

Selective $CD34^+$ cells collected from peripheral blood are one of a graft source for the patients. Positive stem cell selection was first developed for autologous transplantations in high risk patients of refractory and relapsing diseases. Along with the development of selection techniques and devices, it has been used for allogeneic transplantation in leukemia and myelodysplastic syndrome which have high risk factors of GVHD with or without graft failure [8]. This method was used to reduce the risk of GVHD, and similar results were observed in SAA patients, selective $CD34^+$ cell infusion significantly decreased GVHD while slightly raised the risk of graft failure due to small amount of T cells [2,9-11]. To prevent graft failure with lesser risk of GVHD, highly selective and large numbers of $CD34^+$ cell infusion has been shown to be a successful choice [2]. They were also useful for patients who had history of heavy transfusions before transplantation, for patients with unrelated, mismatched or haploidentical donor in SAA. Large numbers of selected stem cells are thought to accelerate immune reconstitution.

Trakhtman et al. [6] reported a SAA patient with graft dysfunction after matched sibling BMT who showed mixed chimerism (95% of donor cell) in BM study, this resulted in reversion to full donor-type chimerism after second infusion of $CD34^+$ enriched peripheral blood cells. The patients experienced acute GVHD in spite of GVHD prophylaxis with mofetil mycophenolate and basiliximab. In this case, boost treatment was performed 3 months after BMT. Chung et al. [12] also reported similar result showing successful peripheral blood stem cell rescue for 7 aplastic anemia patients with late graft failure. Complete donor chimerism was maintained at the time (median 9.2 months from the initial transplantation) of boost treatment. Cyclosporine A was used for GVHD prophylasix although 2 patients developed acute GVHD and 3 patients developed chronic GVHD. Larocca et al. [13] compared trilineage recovery and GVHD among three groups of patients with primary and secondary poor graft function following allogeneic stem cell transplantation. Among patients who received no further infusion, who received a boost of unmanipulated stem cells from original donor, and who received $CD34^+$ selective stem cells, the third group showed significant better tilineage recovery (40% vs. 36% vs. 75%) and low incidence of acute GVHD (0% vs. 29% vs. 0%).

In our two cases, boost treatment using selective $CD34^+$ cells in condition of secondary graft insufficiency showed successful results. BM was replaced with 100% donor cells and the boost treatment was performed 4 to 5 years after BMT as a result of late graft failure. The signs of graft failure were shown earlier after the transplantation (3 months and 11 months after, respectively) however boost treatment was done several years after as the need of transfusion increased. Since the patients showed complete donor chimerism, and $CD34^+$ cell infusion showed lesser risk of acute GVHD [2,10,13], we performed rescue therapy without GVHD prophylaxis and they showed no complications. Due to the short duration of follow-up period, the development of chronic GVHD is not discussed here although it should be monitored since there is higher risk of extensive chronic GVHD following boost cell infusion [12,13].

Few studies have discussed the role of boost treatment of purified selective $CD\ 34^+$ cells in late graft failure after sibling BMT in children with aplastic anemia. This report suggest that selective $CD\ 34^+$ cell boost treatment, without further conditioning or GVHD prophylaxis, can be a promising curative treatment in late graft failure of SAA patients who previously underwent sibling BMT.

References

1. Scheinberg P. Aplastic anemia: therapeutic updates in immunosuppression and transplantation. Hematology Am Soc Hematol Educ Program 2012;2012:292-300.
2. Benesch M, Urban C, Sykora KW, et al. Transplantation of highly purified CD34+ progenitor cells from alternative donors in children with refractory severe aplastic anaemia. Br J Haematol 2004;125:58-63.
3. Eapen M, Davies SM, Ramsay NK. Late graft rejection and second infusion of bone marrow in children with aplastic anaemia. Br J Haematol 1999;104:186-8.
4. Remberger M, Ringden O, Ljungman P, et al. Booster marrow or blood cells for graft failure after allogeneic bone marrow transplantation. Bone Marrow Transplant 1998;22:73-8.
5. Min CK, Kim DW, Lee JW, Min WS, Kim CC. Additional stem cell therapy for graft failure after allogeneic bone marrow transplantation. Acta Haematol 2000;104:185-92.
6. Trakhtman P, Shipicina I, Balashov D, et al. CD34-enriched peripheral stem cells infusion for graft insufficiency treatment in a pediatric patient with severe aplastic anemia after allogeneic bone marrow transplantation. Bone Marrow Transplant 2004;34:465-6.
7. Bin JH, Yoo YK, Kim SY, et al. The Effects of multiple transfusion on the outcomes of bone marrow transplantation from HLA-matched sibling donor in patients with severe aplastic anemia. Korean J Pediatr Hematol Oncol 2003;10:30-8.
8. Markiewicz M, Holowiecki J, Wojnar J, et al. Allogeneic transplantation of selected peripheral CD34+ cells with controlled CD3+ cells add-back in high-risk patients. Transplant Proc 2004;36:3194-9.
9. Cho BS, Eom KS, Kim YJ, et al. HLA-matched sibling transplantation with BM and CD34(+)-purified PBSCs in adult patients with high-risk severe aplastic anemia to overcome graft rejection without an increase in GVHD. Bone Marrow Transplant 2010;45:1497-501.
10. de la Rubia J, Cantero S, Sanz GF, et al. Transplantation of CD34+ selected peripheral blood to HLA-identical sibling patients with aplastic anaemia: results from a single institution. Bone Marrow Transplant 2005;36:325-9.
11. Martin PJ, Hansen JA, Buckner CD, et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood 1985;66:664-72.
12. Chung IJ, Lee JJ, Park MR, et al. Allogeneic peripheral blood stem cell rescue of late graft failure after bone marrow transplantation in patients with aplastic anemia. J Korean Med Sci 2002;17:468-74.
13. Larocca A, Piaggio G, Podesta M, et al. Boost of CD34+-selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation. Haematologica 2006;91:935-40.