



Successful engraftment after infusion of multiple low doses of CD34+ cells from a poorly matched sibling donor in a patient with severe aplastic anemia

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The dose of CD34+ cells is known to influence the outcome of allogeneic peripheral blood stem cell (PBSC) and/or T-cell-depleted transplantation. A previous study proposed that 2×10^6 CD34+ cells/kg is the ideal minimum dose for allogeneic transplantation, although lower doses did not preclude successful therapy. In the case we present here, CD34+ cells were collected from a matched sibling donor on the day of allogeneic hematopoietic stem cell transplantation; however, the number of cells was not sufficient for transplantation. Consequently, PBSCs were collected three additional times and were infused along with cord blood cells from the donor that were cryopreserved at birth. The cumulative dose of total nuclear cells and CD34+ cells was 15.9×10^8 cells/kg and 0.95×10^6 cells/kg, respectively. White blood cells from this patient were engrafted on day 12. In summary, we report successful engraftment after infusion of multiple low doses of CD34+ cells in a patient with severe aplastic anemia.

Keywords: Aplastic anemia; CD34; Cord blood stem cell transplantation; Peripheral blood stem cell transplantation

Introduction

Severe aplastic anemia (SAA) is a rare and fatal disease characterized by immune-mediated dysfunction of hematopoietic stem cells [1]. The development of allogeneic peripheral blood stem cell transplantation (PBSCT) has dramatically increased the success rate of SAA treatment [2]. In histocompatible siblings, PBSCT is an effective treatment for SAA [3,4]. Allogeneic hematopoietic peripheral stem cell transplantation is beneficial to both donors and recipients: the donor avoids the inconvenience and risk of the collection procedure, including general anesthesia; the patient benefits from a more rapid recovery of the hematopoietic and immune systems, which reduces morbidity and promotes early discharge, compared to bone marrow

transplantation [5]. It has been proposed that the minimum number of CD34+ cells in peripheral blood stem cells (PBSCs) required for successful engraftment in allogeneic hematopoietic stem cell transplantation is 2×10^6 cells/kg. Administration of sufficient numbers of CD34+ cells to the recipient is an important prognostic factor and is known to affect transplant-related mortality [6,7]. Here, we report a patient with SAA who received PBSCs from their human leukocyte antigen (HLA)-identical sibling. We were, however, unable to harvest a sufficient number of donor PBSCs for engraftment on the day of transplantation. As a result, multiple collections of donor PBSCs were made and infused into the recipient over 4 additional days, together with the donor's cord blood that was cryopreserved at birth.

Case

A 6-year-old patient was hospitalized due to pancytopenia identified from the results of a complete blood cell count. At the initial diagnosis, laboratory tests showed a leukocyte count of $3.0 \times 10^9/L$, hemoglobin of 8.5 g/dL, platelet count of $19 \times 10^9/L$, absolute neutrophil (ANC) count of $0.84 \times 10^9/L$, and corrected reticulocytes at 1.43%. Furthermore, a bone marrow examination revealed hypocellularity (less than 10% marrow cellularity). After evaluation, the patient was diagnosed with moderate aplastic anemia. In accordance with the criteria of aplastic anemia, immunosuppressive therapy (IST) was performed with anti-thymocyte globulin (ATG), cyclosporine, and prednisolone (PD). The patient was treated with 3.5 mg/kg ATG for 5 days, 7.5 mg PD TID (1 mg/kg) for 5 days, and 180 mg cyclosporine-A (CSA). No IST response was observed after 6 months, and follow-up testing at this time showed a leukocyte count of $1.8 \times 10^9/L$, hemoglobin of 7.1 g/dL, platelet count of $13 \times 10^9/L$, ANC count of $0.52 \times 10^9/L$, and corrected reticulocytes at 0.40%. The patient was diagnosed with SAA, and an allogeneic hematopoietic stem cell transplant was performed 1 month later. Tests for parvovirus and cytomegalovirus (CMV) were both negative, and the patient also tested negative for congenital bone marrow failure syndromes, including Wiskott-Aldrich syndrome and Fanconi anemia.

The selected donor was the patient's 3 year-old brother, who was HLA- and ABO-matched and blood type Rh+ O. He also tested negative for parvovirus and CMV, and there were no unusual findings in the pre-transplantation evaluation. A 10 µg/kg dose of granulocyte colony stimulating factor (G-CSF) was administered subcutaneously to the donor from day -3 to day 0, which increased the donor's white blood cell (WBC) count to $20.9 \times 10^9/L$ at day 0 (reticulocyte 2.38%, ANC $1.525 \times 10^9/L$).

The condition regimen included intravenous (IV) fludarabine ($30 \text{ mg/m}^2/\text{day}$ from day -6 to day -2), cytoxan (25 mg/kg/day from day -6 to day -3), and thymoglobulin (2.5 mg/kg/day from day -3 to day -1). To prevent graft-versus-host disease (GVHD), a 5 mg/kg/day dose of IV CSA was administered beginning the day before transplantation (day -2). Additionally, mycophenolate mofetil was administered orally at a dose of 15-20 mg/kg the day after transplantation.

Hematopoietic stem cells were harvested from the donor after 4 days of G-CSF administration up to the day of transplantation, according to the allogeneic PBSCT protocol. A total volume of 180 mL (total nuclear cell [TNC] 4.89×10^8 cells/kg, CD34+ 0.36×10^6 cells/kg) was harvested, and mobilized infusion was performed during the first treatment. Since the number

of CD34+ cells was much lower than the threshold value, we decided to repeat the PBSCT collection. However, despite performing a total of four PBSC harvests from the donor, the required number of CD34+ cells was not obtained (2nd PBSCT: TNC 3.56×10^8 cells/kg, CD34+ 0.29×10^6 cells/kg; 3rd PBSCT: TNC 4.90×10^8 cells/kg, CD34+ 0.21×10^6 cells/kg; 4th PBSCT: TNC 2.39×10^8 cells/kg, CD34+ 0.06×10^6 cells/kg). As a result, we also administered the donor's cord blood cells that were frozen and stored, followed by cord blood infusion with 0.14×10^8 cells/kg TNC and 0.03×10^6 cells/kg CD34+ cells. The total cell count for the five injections was 15.88×10^8 cells/kg TNC and 0.95×10^6 cells/kg CD34+ (Table 1).

For engraftment, IV administration of G-CSF 250 µg (300 µg/m^2) was started on day 4. Twelve days after the first transplantation, WBC engraftment was achieved with a mean neutrophil count $>0.5 \times 10^9/L$. Platelet engraftment without transfusion for over 2 weeks (platelet count $>20 \times 10^9/L$) was achieved on day 21. The donor chimerism result was completely changed when measured 1 month after the first stem cell infusion. After transplantation, the patient showed no signs of acute/chronic GVHD and was discharged from the hospital. The patient maintained complete remission; bone marrow examination results revealed cellularity above 50%, and the donor chimerism value was maintained for a full year.

Table 1. Five transplantation in 5 days, including cord blood from the donor.

Infusion	Date of infusion (day)	TNC ($\times 10^8/\text{kg}$)	CD34+ cell ($\times 10^6/\text{kg}$)	Stem cell source
1st	0	4.89	0.36	PB
2nd	1	3.56	0.29	PB
3rd	2	4.90	0.21	PB
4th	4	2.39	0.06	PB
5th	5	0.14	0.03	CB
Total cell count		15.88	0.95	

TNC, total nuclear cell; PB, peripheral blood; CB, cord blood.

Discussion

SAA is a heterogeneous disorder of bone marrow stem cells and/or their microenvironment, and can be due to various mechanisms, including autoimmune processes and viral infection [4]. If treated inappropriately, SAA can be a potentially fatal disorder in children. The main approaches to SAA therapy are hematopoietic stem cell transplantation and IST, and for children the current algorithm recommends transplantation, if there is a matched sibling donor. In the absence of a matching donor, IST

using a combination of ATG and cyclosporine is the first choice of treatment [8-10].

The importance of CD34+ cell dose in PBSCT has been clearly demonstrated in autologous or homogeneous peripheral blood hematopoietic stem cell transplantation. CD34 is a type I transmembrane protein expressed by hematopoietic stem cells, and CD34+ cells have the ability to differentiate into all bone marrow cell lineages. Clinically, CD34+ cells are considered equivalent to pluripotent stem cells, with self-renewing capabilities. The dose of injected CD34+ cells is known to be an important factor in autoimmune and allogeneic stem cell transplantation success [11]. The infusion of large numbers of CD34+ cells in PBSCT is thought to accelerate hematopoietic engraftment and reduce graft-related morbidity [12,13].

In a previous study, Singhal et al. recommended a CD34+ cell dose of 2×10^6 cells/kg as the minimum threshold for allogeneic sibling blood or marrow stem cell transplantation [7]. Bittencourt et al. recommended a CD34+ cell dose of 3×10^6 cells/kg as optimal for allogeneic BM transplantation in terms of faster engraftment and decreased treatment-related mortality [6]. In 2010, Islam et al. showed that infusion of less than 2×10^6 CD34+ cells/kg was associated with an increased incidence of graft failures, a higher incidence of bacterial infection, and a delay in neutrophil engraftment [11].

In the present case, we injected 0.36×10^6 cells/kg CD34+ cells, which is much lower than the previously proposed minimal threshold of 2×10^6 CD34+ cells/kg. We administered four PBSC injections with 0.92×10^6 cells/kg CD34+ cells, followed by cord blood infusion with 0.14×10^8 cells/kg TNC and 0.03×10^6 cells/kg CD34+ cells. Despite the fact that these values were lower than the recommended threshold value, ANC engraftment was successful after 12 days without any additional abnormalities, such as GVHD infection. The result of bone marrow and polymerase chain reaction with short tandem repeat markers showed that the patient maintained full donor chimerism at their 1 month and 1 year follow-up examinations. After the first transplantation, it took 12 days to engraft the ANC, 8 days after the fourth PBSC infusion, and 7 days after the cord blood transplant. Additionally, de la Rubia et al. showed that the median time taken to achieve engraftment with neutrophil counts $>0.5 \times 10^9/L$ was 12 days (range, 9–13 days) [14]. From this, the first peripherally infused stem cells (CD34+ 0.36×10^6 cells/kg) were considered to have a major influence on engraftment in the present case.

In addition, the donor cord blood cell infusion, which was performed last, is known to contain mesenchymal stem cells (MSCs), which improve the recovery of hematopoietic

function and prevents GVHD following hematopoietic stem cell transplantation (HSCT). MSCs are a type of adult stem cell found in many tissues and organs. They are capable of self-renewal, multiple screening, and regulation of immune function. MSCs are progenitors of bone marrow stroma, and MSCs isolated from bone marrow, blood, and cord blood have been shown to promote engraftment after hematopoietic stem cell transplantation. Moreover, MSCs facilitate engraftment of neutrophils and platelets, and contribute to 100% donor chimerism. MSCs in HSCT can promote stem cell transplantation, improve hematopoietic function recovery, and prevent GVHD [15]. In the present case, it is possible that MSCs facilitated the engraftment of neutrophils and platelets contained in the administered peripheral blood and cord blood stem cells. Additionally, cord blood stem cell infusion may have supplemented the otherwise small CD34+ cell dose. The reason for the insufficient number of CD34+ cells harvested from the donor is not clear. The donor's body weight was 16.9 kg, which is less than the patient, and could be one of the causes of the insufficient harvest. We attempted to further evaluate the donor, but his mother refused.

In conclusion, we report successful engraftment after infusion of multiple low doses of CD34+ cells from a poorly matched sibling donor in a patient with SAA.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported

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