Arch Immunol Ther Exp, 2005, 53, 364–368
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Original Article

Received: 2004.11.17
Accepted: 2005.05.05
Published: 2005.08.15

Simultaneous transplantation of two allogeneic units of cord blood in an adult patient with acute myeloblastic leukemia. A case report

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: by the State Committee for Scientific Research (KBN, Poland) grant No. PBZ-KBN-083/P05/2002.

Summary

Introduction: The major obstacle to the therapeutic use of hematopoietic transplantation is the unavailability of matched, unrelated marrow donors for the large number of potential patients, although all of them have the chance to find sufficiently matched, unrelated cord blood units. However, the use of cord blood as a source of cells for transplantation is limited by its cell number, usually below 1 billion, which allows for routine transplantation only in children weighting less than 30 kg, while most potential recipients possess a higher body mass. This led to the idea of the simultaneous use of several units of cord blood which, combined, would fulfill the requirements for the necessary cell number for an adult recipient.

Materials and Methods: We attempted to simultaneously transplant an adult patient with refractory acute myeloblastic leukemia utilizing two different cord blood units, one fully matched and one mismatched at one locus.

Results: The patient became reconstituted with only one unit, the mismatched, as determined using microsatellite markers, and had no signs of relapse of leukemia. Unfortunately, he died of persistent fungal (brain aspergilloma) infection on day +103.

Conclusion: The successful engraftment may suggest that a method based on the principle of using more than one cord blood unit for transplantation is feasible in large adult patients and may reach routine application.

Key words: placental blood • bone marrow transplantation • hematopoiesis

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INTRODUCTION

Adult patients suffering from diseases that are indications for the transplantation of hematopoietic cells suffer from a shortage of available donors. Particularly patients with rare combinations of HLA antigens cannot find an appropriate match in registries of unrelated donors. One possible solution is to use cord blood, which gives similarly satisfactory results whether transplanted at a 6/6, 5/6, or 4/6 antigen match[2, 4, 6]. This is most likely due to the immune system's greater plasticity in developing newborn stem cells of cord blood compared with those of marrow and adult stem cells of an unrelated bone-marrow/peripheral-blood stem cell donor. However, the main obstacle to the use of cord blood in adult patients is the limited volume (and, subsequently, cell number), usually sufficient for recipients not exceeding 30 kg in body weight[5, 10, 13]. There are two possible ways to deal with this problem: explore the possibility of *ex vivo* stem cell expansion[3, 9] or simultaneously transplant more than one unit of cord blood[1, 6]. We became interested in this second option.

Below we report a case of resistant acute myeloblastic leukemia transplanted with two units of cord blood.

MATERIALS AND METHODS

A 21-year-old male was diagnosed with acute leukemia (M4) in October 2002. He received standard induction chemotherapy (daunorubicine, 60 mg/m^2 days 1–3, plus cytosine arabinoside, 200 mg/m^2 days 1–7) followed by three subsequent courses of reinduction chemotherapy (where daunorubicin was replaced by either idarubicin or mitoxantrone, and cladribine was added). He achieved only partial remission, maintaining 60% of leukemic blasts in his bone marrow, though he was still in relatively good clinical condition (Karnofsky 80%). He had no siblings, and the search for an unrelated marrow donor also failed. Unexpectedly, we found in our department's cord blood bank (which is relatively small) three units that partially matched him. One unit was disqualified because of its very small volume, and two units were finally selected, one matched at 6 and the other at 5 out of 6 antigens for which the units had been pretested (Table 1). There was also a major AB0 mismatch between the recipient and each of the units, and between the units themselves. There was the possibility to evaluate post-transplantation hematopoietic chimerism studies (using an earlier described method[8]) because two microsatellite markers (FGA and D81179) were informative, that is they differentiated the recipient and each of the two units selected for transplantation. The treatment protocol was accepted by the Bioethics Commission of the Medical University of Warsaw and the patient signed informed consent.

The patient was transplanted on May 16, 2003, following conditioning with 16 mg/kg of busulphan and 200 mg/kg of cyclophosphamide as well as with 3.75 mg/kg of thymoglobuline on days –4 and –3 pre-transplantation and on days +7, +9, and +11 post-transplantation. Since the patient had a body weight of 85 kg, the total number of transplanted cells was 2.2×10^7/kg and the number of CD34$^+$ cells was 0.5×10^6/kg. The number of BFU-E was 2.2×10^4/kg and that of GM-CFU was 2.25×10^4/kg. Graft-versus-host prophylaxis included cyclosporin A beginning from day –1, initially 5 mg/kg i.v. and then lowered to maintain a blood level of 200–300 μg/ml.

Table 1. HLA and major blood group types for the recipient and the donor cord blood units as well as body weight and nucleated cell numbers

Subject	Body weight (kg)/total nucleated cell number (10^6)	Blood group	HLA-A	HLA-B	HLA- Cw	HLA- DRB1	HLA- DQB1
Recipient UBMID 133	85	0 Rh(+)	1 1	8 57	7 6	*03 *15	*02 *06
Cord blood unit no. 1 (UCBU 14)	910	A Rh(–)	01 01	0801 5701	0602 0701	*0301 *1501	0201 0602
Cord blood unit no. 2 (UCBU 51)	920	B Rh(+)	01 01	0801 5701	0602 0701	*0301 ***0701**	0201 06xx
Recipient on day +100 after transplantation		not tested	01	08 57		03 **07**	

In bold is the antigen unique to cord blood unit no. 2. UBMID – unique bone marrow identification, UCBU – unique cord blood unit (these numbers refer to the obligation of the transplant unit to a carry register of the unique numbers of all patients treated).

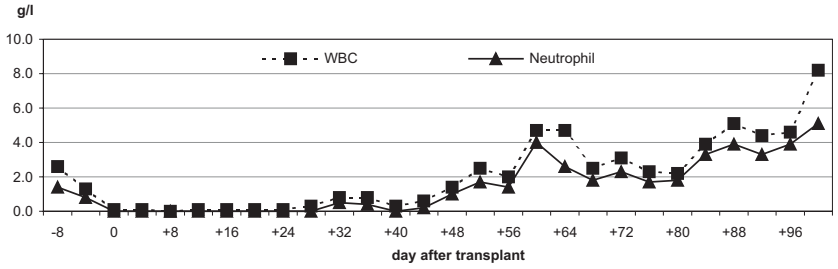

Figure 1. Recovery of white blood cell (WBC) and neutrophil counts in an adult patient transplanted with two units of cord blood.

The post-transplant course was extremely complicated, with signs of pulmonary aspergillosis observed on day +6, bacterial (*Enterococcus*) sepsis on day +12, graft-versus-host disease on day +10, and hemorrhagic cystitis starting on day +18. The patient was treated with amphotericin B and flucytosine, with partial resolution of pulmonary lesions. Signs similar to graft-versus-host disease stage II (skin rash and hyperbilirubinemia) were apparent on day +10 and resolved spontaneously on cyclosporine prophylaxis on day +14. No biopsy was taken to confirm this suggestion. Bacterial sepsis resolved after linezolide treatment. Hemorrhagic cystitis was treated with argentum nitricum, prostaglandin α washes and formalin washes, and was associated with significant blood losses and resolved on day +86. The patient also received ganciclovir because of CMV reactivation detected by PCR testing. Beginning on day +81, pulmonary changes gradually started to progress again and were joined by behavioral abnormalities, which, combined, led to the diagnosis of brain and lung aspergillosis. These changes progressed and the patient died on day +103.

RESULTS

The recovery of the neutrophil count started on day +32 and the patient reached 1.5×10^9/l on day +52 (Fig. 1). While the platelet count did not increase significantly, there was a reduction in the requirements for platelet transfusion. Red blood cell transfusions were required almost the whole time, having to compensate not only for the absence of red cell production, but also for very significant blood loss (up to 500 ml per day) due to hemorrhagic cystitis. Bone marrow aspiration on day +100 revealed normal cellularity of the bone marrow, with trilineage hematopoiesis and absence of leukemia.

Analysis of both bone marrow and peripheral blood performed on day +100 revealed complete chimerism with the engraftment of only one unit, which was unit 2 (Fig. 2). Unexpectedly, it was the unit that was partially HLA-mismatched and not the one that was fully matched. This was further confirmed by the results of HLA typing of the recipient performed on day +100 showing the presence of DBR1 07, which was unique to unit 2 (Table 1).

Autopsy revealed: foci of necrotizing aspergillosis in the lungs, thyroid gland, and brain (cerebellum), moderate signs of hemorrhagic diathesis (mucosal and serosal petechiae), and hemorrhagic urocystitis, The bone marrow was moderately cellular, with trilineage (erythrocytic, granulocytic, megakaryotic) normal hematopoiesis, and no signs of leukemia recurrence.

DISCUSSION

The post-transplant course in this patient confirmed that simultaneous transplantation of two cord blood units is feasible and can lead to the restoration of normal hematopoiesis. In agreement with earlier sug-

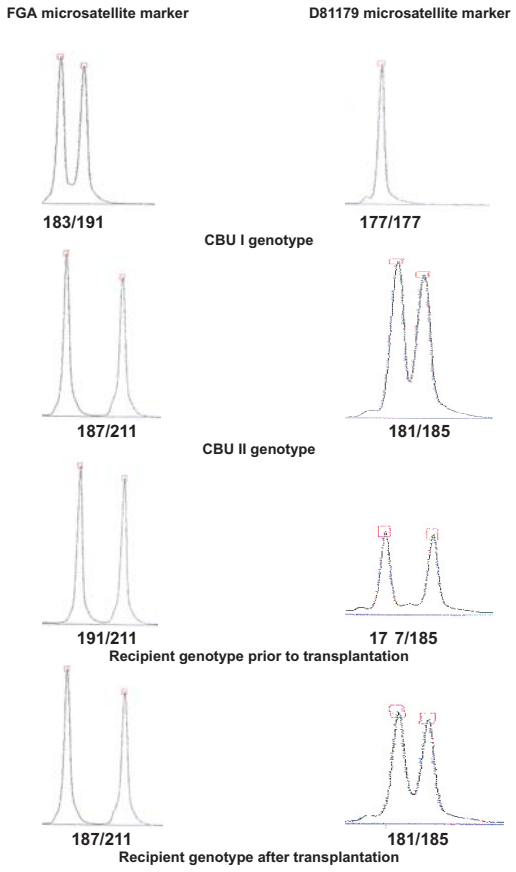

Figure 2. Post-transplant chimerism analysis using microsatellite markers FGA and D81179. The numbers under the curves indicate the base pairs at which the particular peaks were found on electrophoresis.

gestions by Barker et al.[1] that recipients of two cord blood units become reconstituted with the progeny of cells derived from only one of these units, this patient was also reconstituted by only one of the transplanted units. This may raise the question whether it is at all necessary to transplant more than one unit of cord blood in an adult recipient. In fact, in the mouse it is possible to reconstitute an adult recipient with the progeny of only one hematopoietic stem cell[14]. Cord blood represents approximately one half of the entire peripheral blood of the newborn, and from the biological point of view it should contain a sufficient number of stem cells to reconstitute hematopoiesis in any human organism. Furthermore, in this case the kinetics of reconstitution shown in Fig. 1 was not different from that reported for the transplantation of a single unit.

However, while studies analyzing cord blood transplants have shown that the average threshold of the number of cells required is in fact much lower for cord blood than for bone marrow (37×10^6/kg for cord blood versus $100–200\times10^6$/kg for bone marrow), they have simultaneously shown that the number of transplanted cells is the single most important factor predicting the outcome of transplantation, more important than the HLA compatibility for up to 2 of 6 antigens, with a high transplant rejection rate when this threshold is not reached[5, 13]. This was the basis for the idea of simultaneously transplanting multiple cord blood units.

From the biological point of view, such a transplantation creates novel problems, such as the interaction of the hematopoietic stem cells and their progeny from one unit not only with the recipient, but also with the hematopoietic stem cells and their progeny from the other unit (units). This may lead to mutual inactivation[11, 12], competition, and tolerance. The phenomenon of stem cell inactivation in the situation of the simultaneous transplantation of histo-incompatible hematopoietic stem cells was described many years ago and has not been studied further. Since this phenomenon is due to the activity of immune cells accompanying the stem cells in the transplant, it may not take place during the transplantation of cord blood, which contains more immature (and presumably less reactive) immune cells than adult marrow. On the other hand, it is this phenomenon that may be responsible for the observed hematopoietic reconstitution being derived from only one unit in the recipients of multiple units. If this explanation is true, then after the transplantation of more than one cord blood unit, the immune cells fight each other and the recipient is repopulated by the progeny of the "winning" unit. However, it is also possible that all that goes on is a simple competition of the transplanted stem cells for the recipient, who becomes repopulated with the progeny of the stem cells with the greatest proliferative potential. In such a case, the transplantation of stem cells derived from more than one donor just serves the purpose of increasing the probability of having among the transplanted cells ones with higher proliferative potential. There is definitely an urgent need for more studies exploring the biological phenomena that occur during the transplantation of multiple cord blood units.

The fact that the post-transplant course in this case had so many complications was probably not related to the source of the transplanted cells, but rather to the pre-transplant state of the recipient, who was heavily pretreated and with resistant leukemia. Moreover, he had most likely been already infected with aspergillus prior to the transplant, but this infection was sub-clinical. From this point of view, that the patient survived so long despite so many life-threatening complications has to be attributed to the activity of the transplant, which was providing cells helping to control these complications.

This is still one of the first descriptions of an outcome of such a procedure. A report of 23 transplanted cases by Barker et al.[1] described a 33% success rate, and there is a report of one successfully transplanted case from China[7].

In summary, the post-transplant course in this case combined with the aforementioned reports of other authors supports the notion that the simultaneous transplantation of more than one unit of cord blood may be a practical solution in the treatment of adult patients who lack a marrow donor.

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