New insights into cord blood stem cell transplantation

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Purpose of review

To review the available clinical and biological advances of umbilical cord blood allogeneic stem cell transplantation in pediatric and adult patients.

Recent findings

Recent large international studies suggested that allogeneic umbilical cord blood transplantation may potentially emerge as the frontline stem cell source for pediatric patients with hematopoietic malignancies because of its ability to confer superior overall and relapse-free survival compared with matched marrow stem cells. In adults, umbilical cord blood transplantation, double umbilical cord blood units and nonmyeloablative engraftment strategies have attracted further attention in clinical practice with the advantages of possible stronger graft-versus-leukemia effect and expanding transplantation indications. Additional advances in the basic biology of umbilical cord blood also appear very promising in development of enhanced engraftment approaches for limiting hematopoietic stem cell numbers or expansion of repopulating cells.

Summary

Umbilical cord blood is a valuable alternative source of hematopoietic stem cells for patients that require allogeneic transplantation in the absence of readily available human leukocyte antigen matched marrow or blood hematopoietic stem cells. The current advances in clinical and biological research will further expand its application in pediatric and adult hematopoietic stem cells transplantation for treating hematologic disorders.

Keywords

allogeneic stem cell transplant, graft-versus-host disease, hematopoietic stem cell, pediatric leukemia, umbilical cord blood, xenotransplantation

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Introduction

Since Gluckman *et al.* [1] collectively performed the first umbilical cord blood (UCB) transplantation to treat a child with Fanconi's anemia in October 1989, UCB has been widely accepted as an important alternative hematopoietic stem cells (HSCs) source for patients who have no readily available human leukocyte antigen (HLA) matched HSC donors and require HSC transplantation for their underlying medical conditions. To date, more than 10 000 UCB transplant procedures have been performed worldwide for pediatric [2–8] and adult patients [5,9–14]. Compared with peripheral blood stem cell (PBSC) and marrow HSC, UCB has many compelling advantages as an attractive HSC source for transplantation purposes (Table 1).

Every year, the number of UCB transplants is rapidly increasing both in pediatric (Fig. 1a) and adult (Fig. 1b) patients. Owing to the less stringent HLA matching requirements between donor–recipient, nearly all (> 95%) patients are able to find at least one potential 4–6/6 matched UCB unit on the National Marrow Donor Program (NMDP) Registry and the majority will find a potential 5/6 match (http://www.marrow.org/PHYSICIAN/URD_Search_and_Tx/Likelihood_of_Finding_an_URD_o/index.html). Recipients who received 4–5/6 HLA matched UCB unit(s) have shown graft-versus-host disease (GVHD) rates and survival outcomes in pediatric and adult patients similar to those who receive 6/6 HLA matched unrelated donor (MUD) marrow transplants [15–17]. We anticipate that UCB transplantation will continue to grow and will attract more attention in clinical and biological research in the future. This growth will also be stimulated by advances in the basic biology of UCB and new approaches for expansion of absolute HSC numbers and improvement of engraftment ability.

Recent clinical advances in umbilical cord blood transplantation

Eapen *et al.* [16] recently conducted a comprehensive comparison study through collaborative efforts between

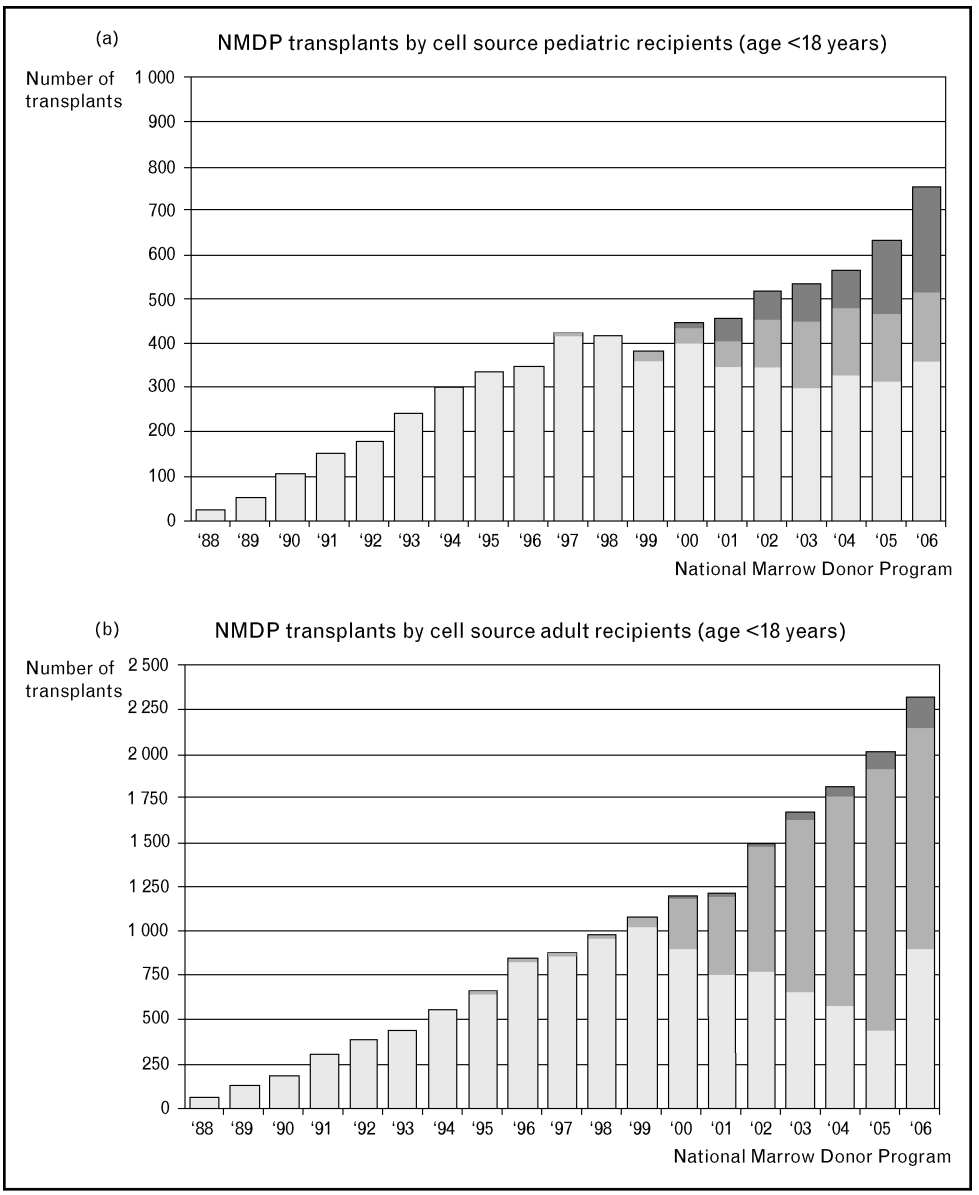
Table 1 Advantages of UCB as hematopoietic stem cells for allogeneic transplantation

Umbilical cord blood is harvested with no risk to the mother or infant.
UCB is not associated with current ethical concerns raised in use of embryonic stem cells because the cells are collected after delivery of a full-term normal infant.
Ethnic balance in a cord blood repository can be reached and maintained with targeted collection programs.
The chance of viral contamination of UCB from cytomegalovirus and Epstein Barr virus is low due to minimal placental transmission rates.
UCB units from banks can be made immediately available to patients who need emergent allogeneic stem cell transplantation.
Frozen UCB can be easily shipped and thawed for use when needed, compared to freshly donated bone marrow which has a limited shelf-life, necessitating coordination between harvesting physicians, transportation personnel, and transplantation teams.
Stored UCB suffers no attrition except by clinical use resulting in an undistorted accumulation of HLA genotypes.

HLA, human leukocyte antigen; UCB, umbilical cord blood.

New York Blood Center (NYBC) and the Center for International Blood and Marrow Transplantation Research (CIBMTR), comparing the outcomes of children with acute leukemia who received HLA matched and mismatched UCB ($n = 503$) or 8/8 allele HLA matched MUD marrow ($n = 116$). All children were younger than 16 years undergoing myeloablative transplantation. The UCB recipients tended to be younger

Figure 1 Diseases treated by umbilical cord blood transplant



(a) Trend in treatment of pediatric disease treated by UCB transplant. (b) Trend in treatment of adult disease by UCB transplant. Data are obtained from the National Marrow Donor Program. NMDP, National Marrow Donor Program; PBSC, peripheral blood stem cell; UCB, umbilical cord blood.

in age, more likely nonwhite, in relapse before transplantation, and those who received HLA mismatched grafts.

The median times to neutrophil and platelet recovery were slower in UCB recipients (25 and 59 days) compared with marrow recipients (19 and 27 days). The probability of neutrophil recovery at day 42 was lower in UCB mismatched recipients regardless of the graft cell dose ($P < 0.0001$) compared with marrow recipients. However, the probability of neutrophil recovery was not different ($P = 0.0626$) between matched UCB recipients and marrow recipients (HLA matched and mismatched). In comparison with allele matched bone marrow transplants, 5-year leukemia-free survival (LFS) was similar to that of after transplants with UCB HLA mismatched at one or two loci with potentially superior results in recipients of HLA matched UCB. For recipients of 8/8 allele HLA matched marrow, mismatched marrow, 6/6 antigen HLA matched UCB, 5/6 antigen HLA matched UCB (cell dose $>3.0 \times 10^7$ NC/kg) and 4/6 antigen HLA matched UCB (any cell dose), the adjusted LFS rates were 38, 37, 60, 45, and 33%. These data from the NYBC and the CIBMTR provocatively suggest that HLA matched or high dose mismatched UCB transplantation can potentially be a front line therapy for pediatric acute leukemia patients even when HLA matched marrow donors are available.

Double umbilical cord blood transplantation

The Minnesota group [18,19] has pioneered utilization of double UCB grafts in order to overcome the cell dose limitation in for larger patients. Brunstein *et al.* [20] recently updated their largest single institution experience in nearly 200 double UCB transplantation in the myeloablative and nonablative settings. The data included advanced stage hematologic malignancies in pediatric and adult patients and indicated that double UCB transplantation is technically feasible, clinically safe, and increased eligibility of adult patients for transplantation. The overall clinical outcomes (incidence of GVHD and survival) were comparable in patients that received double versus single UCB transplantation in a myeloablative setting. In analyzing the clinical outcomes in acute leukemia UCB recipients, authors found that acute leukemia patients who received double UCB transplantation had a 10-fold decrease in the risk of leukemia relapse compared with patients who received single UCB transplantation. This might be attributed to a relatively higher degree of HLA mismatch among double UCB recipients, of whom 80% at least one UCB unit with two antigens mismatched. A randomized clinical trial has been designed to further confirm the clinical merit of double UCB transplantation in acute leukemia patients.

Nonmyeloablative umbilical cord blood transplantation

Elderly patients with significant comorbidities and patients that relapsed after extensive chemotherapies or prior stem cell transplantation may have unacceptable peritransplantation risk. Again, Brunstein *et al.* [17] from the Minnesota group recently reported on 110 adult patients with hematologic diseases. The preparative regimens were largely low dose total body irradiation (TBI)/fludarabine/antithymocyte globulin (ATG) based and the target UCB cell dose was 3.0×10^7/kg. About 85% patients needed to receive a second partially HLA matched UCB. Neutrophil recovery was 92% at a median of 12 days. Incidences of grades III and IV acute and chronic GVHD were 22 and 23%, respectively. Transplantation-related mortality was 26% at 3 years. Overall and event-free survival (EFS) was 45 and 38%, respectively. Absence of high-risk clinical features and severe GVHD were favorable factors for overall survival (OS) ($P < 0.01$) whereas absence of high-risk clinical feature and double UCB recipients are favorable factors for EFS ($P < 0.01$). They made a similar observation that double UCB recipients had lower relapse rate or EFS.

Another study was reported by Miyakoshi *et al.* [21] in mostly adult acute myeloid leukemia (AML) patients who were conditioned by nonmyeloablative regimen (fludarabine/melphalan/low-dose TBI). The 1 year OS was 33% with a relapse rate of 11%. In general, the nonmyeloablative UCB data are less well defined compared with the myeloablative UCB transplantation.

Umbilical cord blood graft selection

Gluckman *et al.* [14] summarized the UCB unit selective criteria based on current available scientific and clinical data. In general, patients and UCB units must be at least 4/6 HLA matched in A, B, and DR beta 1 (DRB1) loci with possible cell dose more than $2.5–3.0 \times 10^7$ cells/kg for single unit UCB transplantation [22]. Regarding the degree of HLA disparity, priority should be given for selecting UCB grafts in the following order: 6/6 > 5/6 > 4/6. If multiple UCB units with the same level of HLA match are available, the priority should be given to the UCB units that are DRB1 matched with higher cell doses because recipients seemed to have a better OS and disease-free survival (DFS) (personal communication with Dr Cladd Stevens, NYBC).

In patients with malignant disease, graft-versus-leukemia (GVL) appears to be enhanced in recipients of two units. For this reason, it could be argued that such patients should be offered two units even if a single unit exists with the cell doses described above. In patients with nonmalignant disease, a better match as well as higher cell dose unit was associated with less transplant related mortality. As risk of GVHD is higher in recipients of two

units, it could be argued that such patients should preferentially be offered a large single unit if it is available.

It has been known that in more than 95% of recipients of two UCB units, only one unit will contribute to hematopoiesis by day 100 regardless of type of preparative therapy (myeloablative versus nonmyeloablative therapy). However, choosing two UCB units for transplant recipients is complicated because there is no way to predict which unit will be the predominate graft. Despite unproven assumptions, Brunstein and Wagner developed the following algorithm for selecting the two UCB units at the University of Minnesota.

Step 1: Unit 1 is the 'best' single unit that is available – having the best HLA match followed by the best cell dose.

Step 2: Unit 2 is the second 'best' unit that is available that not only has the best HLA match and best cell dose for the recipient but one that is also partially HLA matched with unit 1.

The best unit is defined in order of priority (1–15 below, see Table 2).

It is important to point out that unit 2 is often not the next best unit because of the intraunit matching requirement. In addition, there are other factors that may influence the final selection of units 1 and 2, these include; attributes of the cord blood bank (i.e. some cord blood banks do not meet all the criteria of the Minnesota Programme, some banks have a track record of slow response time and poor service); lack of infectious disease serology; lack of attached segments for quality control testing (i.e. proof of unit identity and HLA type); high cost (i.e. poor exchange rates, payment requirement prior to unit confirmation); high numbers of nucleated red cells; positive cultures. Selection of the two units has become easier with the advent of the NMDP cord blood search tools.

Umbilical cord blood stem cell biology

In parallel with clinical application of UCB for treatment of hematologic malignancies, there have been a number of important advances in understanding the basic biology of UCB. As UCB stem cell numbers are limiting, a major goal has been to explore methods of expansion *in vitro* to increase HSC numbers for transplantation. One approach has been to perform ex-vivo expansion of UCB using innovative nanofiber scaffolds [23]. These have been shown to provide key adhesive surfaces that promote expansion of cryopreserved UCB in the presence of recombinant human stem cell factor (SCF), Flt-3 ligand (Flt3L), thrombopoietin (TPO), and interleukin 3 (IL-3). However, even with the proper interactions there is a need to better understand the signals controlling HSC function. To better understand the critical signaling pathways that are active in UCB HSC may permit better targeting of these in expansion cocktails. Comparison of the expression of 10 000 genes uncovered an increased nuclear factor-kappaB (NF-κB) signaling pathway in UCB resulting in increased expression of transcriptional targets [24]. This distinctive feature of UCB $CD34^+$ cells may provide a starting point for manipulation of UCB prior to transplant. Another recent study showed that angiopoietin-like 5 and insulin growth factor binding protein 2 (IGFBP2) could net an approximately 20-fold expansion of human UCB repopulating activity in nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice [25•].

A major thrust of new discoveries in the UCB research field has come in direct applications to improve transplantation efficiency. This has been approached through several innovative chemical and cellular routes. One study demonstrated that serotonin enhanced expansion of multipotential and myeloid colony-forming cells in methylcellulose cultures and improved engraftment of human $CD45^+$, $CD33^+$, $CD14^+$ cells, BFU/CFU-E, and CFU-GEMM engraftment in the bone marrow of animals 6 weeks posttransplantation [26]. Christopherson *et al.* [27] first reported the critical importance of CD26 as a negative regulator of murine CXC chemokine receptor 4 (CXCR4) activation and showed that inhibition of CD26 prior to transplant markedly improved hematopoietic reconstitution. Now they have reported in two separate papers follow-up to the initial work showing that CD26 inhibition can be effective in promoting long-term xenotransplantation of UCB stem cells into NOD/SCID mice [28,29]. These two studies suggest that the findings observed in the mouse may be translatable into optimized human HSC engraftment. Another approach to understanding UCB engraftment analyzed the NOD genetic background in mice to determine the genetic cause for

Table 2 The best UCB unit selection priority

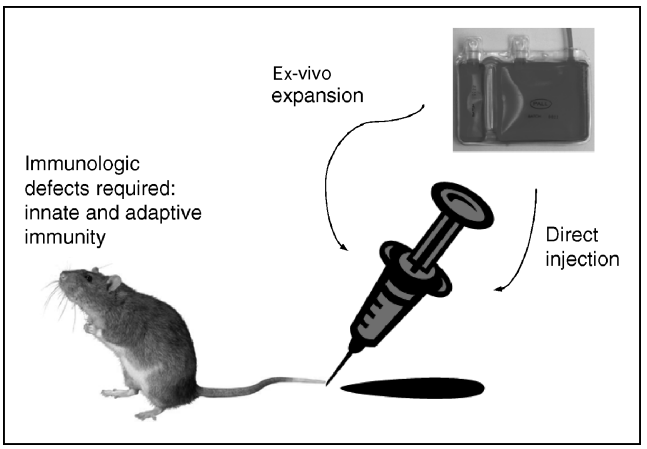
	Cell dose levels						
HLA match	1.5–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0
HLA 6/6	3	2	1				
HLA 5/6	8	7	6	5	4		
HLA 4/6	15	14	13	12	11	10	9

Based on the HLA matching status and cell dose of a given UCB unit, the priority score is listed from 1 to 15. '1' is the highest priority and '15' is the lowest priority. HLA, human leukocyte antigen; UCB, umbilical cord blood.

the improved xenotransplant ability in this host. Interestingly, it was observed that the NOD Sirpa allele was responsible for this improved engraftment through enhanced binding to CD47 [30•]. Since CD47 plays a major role in macrophage function, this study highlights a major role of the adaptive immune system in permitting UCB transplant success. This finding brings up questions about xenotransplants in general and opens up a debate regarding this assay as a measure of true HSC/progenitor function versus a measure of immunologic escape from rejection. Engraftment in the model is likely influenced by both parameters (Fig. 2).

In addition to treatment of the UCB stem cells with novel agents, another approach has been ex-vivo expansion under the support of mesenchymal stem cells (MSCs). In this case, the degree of expansion of white blood cells was enhanced and these expanded cells retained the ability to engraft into NOD/SCID mice; however, definitive proof for quantitative expansion of the SCID mouse repopulating cell (SRC) was not obtained [31]. A different study used a similar approach for expansion and it was reported that total nucleated cells, $CD34^+$ cells and colony forming units in culture (CFU-C) were increased relative to UCB cultured without a MSC feeder layer [32]. However, in the NOD/SCID transplant assay, there was no evidence for expansion of the SRC. Collectively, these new studies combined with prior work in this area demonstrate that MSCs may provide some support for hematopoiesis *in vitro* but are not sufficient to support self-renewal and expansion of the SRC component of the UCB graft.

Figure 2 Xenotransplantation assay for umbilical cord blood stem cells



The NOD/SCID repopulating assays has become accepted as a surrogate for human hematopoietic stem cells. The assay requires adequate suppression of the adaptive and innate immune response to prevent rejection of the donor human cells by the mouse immune system. While greater degrees of immunodeficiency provide more sensitive host mice for transplant assays, the ability of a particular stem cell population to evade the immune response carries a lot of weight in the assay. NOD/SCID, nonobese diabetic/severe combined immunodeficient.

These results do not mean that MSCs or other multipotent components of the UCB graft are not without positive benefits on SRC engraftment. Unrestricted somatic stem cells (USSCs) have recently been identified from within UCB and these cells possess potential to develop into mesodermal, endodermal, and ectodermal fates. Interestingly, USSCs cotransplantation improves the homing of $CD34^+$ cells at 6 weeks posttransplant [33]. These investigators demonstrated further that knockdown of stromal cell-derived factor-1alpha (SDF-1α) using a lentiviral small interfering RNA (siRNA) inhibits this homing response. Therefore, the clinical potential for improving UCB engraftment may be significant when combined with major trophic effects in a co-transplantation setting.

It also merits noting that UCB xenotransplant models present potential platforms for understanding leukemic stem cell biology. Recent work by John Dick has demonstrated that development of leukemogenesis models is feasible. His group first showed that the leukemic fusion protein TEL-JAK2 can be introduced into UCBs using a viral vector approach and that this recapitulates the development of myelofibrosis, which is observed as a complication of polycythemia vera (P. vera) [34]. Since P. vera is characterized by almost exclusive mutation in the JAK2 pseudokinase domain, this model provides a human xenotransplant setting to understand how this erythrocytosis progresses into myelofibrosis. In addition to a model for human chronic myeloproliferative disease, the same group also developed models for mixed lineage leukemia (MLL) fusion proteins that are capable of generating myeloid or lymphoid acute leukemias [35]. Outside of the debate regarding leukemic stem cell frequencies and definition, these models will be ideal for the study of mechanisms of leukemogenesis in human cells and for directly testing novel therapeutics.

Conclusion

UCB units have tremendous promise for treating hematologic disease because of many favorable attributes, including reduced GVHD. Recent advances in UCB stem cell biology, as well as transcription factors that regulate UCB graft T-cell function, UCB homing properties, and assessment of T-cell reconstitution during immune recovery, link between basic principles of stem cell and T-cell biology. The major limitation of reduced numbers of HSCs in UCB is being addressed by basic research. It is promising that potential improvements in engraftment efficiency without increased stem cell numbers or actual increased stem cell numbers through dual UCB transplant or ex-vivo expansion might lead to improved treatment approaches. In summary, hematology patients requiring allogeneic stem cell transplant for treatment of life-threatening blood disorders are already benefiting from UCB transplant and further advances are eagerly anticipated.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 427).

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