Biology of Blood and Marrow Transplantation 7:633-645 (2001)

REVIEW ARTICLE

Current Status of Cord Blood Banking and Transplantation in the United States and Europe

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Received May 16, 2001; accepted September 11, 2001

ABSTRACT

Cord blood (CB) transplantation has expanded the ability of the transplantation community to meet the growing needs of their patients. Clinical data over the last decade show promising results in CB transplantation using blood from related as well as unrelated donors. Basic science continues to look for ways to expand the quality and quantity of CB. CB banks are now established around the world, with major efforts to standardize banking to facilitate regulation, collection, processing, and distribution as a way of providing the highest-quality CB for patient use. This review article discusses the current status of CB transplantation and banking in the United States and Europe.

KEY WORDS

Cord blood transplantation • Blood banks

INTRODUCTION

Since the first successful transplantation using umbilical cord blood (CB) to treat a patient with Fanconi's anemia in 1988 [1], CB transplantation has generated strong interest as an alternative to bone marrow transplantation (BMT) to treat a variety of diseases. Basic research has shown the valuable properties of CB that make it not only a viable alternative but a preferred one for many patients. Clinical studies support this research through trials that show both the efficacy and accessibility of CB for clinical use [2-6].

As interest in and clinical use of CB increases, several important issues have moved to the forefront. Among these are questions about the biological and immunological properties of CB, practical ways to harvest and store CB in banks, administrative questions on how to create and introduce standards into CB banking, and ethical concerns. (These questions and issues were discussed at a symposium held in San Francisco on December 1, 2000, at which international experts presented the most current information on CB banking and transplantation. The symposium was chaired by Jeffrey McCullough, MD, University of Minnesota Medical School. Presenters included Karen Ballen, MD [American Red Cross], Hal E. Broxmeyer, PhD [Indiana University School of Medicine], Wanda Piacibello, MD [University of Torino Medical School], Paolo Rebulla [Milano Cord Blood Bank], Catherine M. Verfaillie, MD [University of Minnesota Medical School], and John E. Wagner, MD [University of Minnesota Medical School].)

CLINICAL STATUS OF CB TRANSPLANTATION

CB transplantation offers the potential to increase the availability of blood to treat a variety of diseases and has shown several advantages over allogeneic BMT: (1) immediate availability, (2) less HLA restriction for donors, (3) lower risk of viral contamination of the graft, and (4) potentially reduced risk of graft-versus-host disease (GVHD) [7]. Currently, there have been more than 1000 CB transplantations performed worldwide from related and unrelated donors to treat patients with malignant and nonmalignant diseases [7,8] (Table 1).

Table 1. *Diseases Treated by Cord Blood Transplantation**

Malignant diseases
Acute lymphocytic leukemia
Acute myelocytic leukemia
Chronic myelogeneous leukemia
Juvenile chronic myelogeneous leukemia
Myelodysplastic syndrome
Neuroblastoma
Nonmalignant Diseases
Adrenoleukodystrophy
Amegakaryocytic thrombocytopenia
Blackfan-Diamond syndrome
Dyskeratosis congenita
Fanconi's anemia
Globoid cell leukodystrophy
Gunther disease
Hunter syndrome
Hurler syndrome
Idiopathic aplastic anemia
Kostman syndrome
Lesch-Nyhan syndrome
Osteopetrosis
Severe combined immune deficiency
Thalassemia
X-linked lymphoproliferative syndrome

*Adapted from Fasouliotis and Schenker [8].

Clinical results of related and unrelated donor CB transplantation are now available from many institutions worldwide. These studies have examined the effect of patient characteristics (eg, age, weight, disease) and donor blood characteristics (eg, HLA match, number of nucleated cells transplanted) on survival, disease-free survival, and engraftment (neutrophil and platelet). Important sources of these clinical outcomes come from CB registries that contain data on combined results from many institutions. For related donor CB transplantations, data from the International Cord Blood Transplant Registry and the Eurocord Registry provide combined results on clinical outcomes. For unrelated donor CB transplantations, two primary registries include the New York Blood Bank and the Eurocord Registry.

CB Transplantation From a Related (Sibling) Donor

The first successful transplantation of CB from a related donor was performed in 1988 to treat a patient with Fanconi's anemia [1]. A more recent case in 2001 of a successful CB transplantation in a 6-year-old girl with Fanconi's anemia treated at the University of Minnesota with CB that was a selected product of in vitro fertilization and preimplantation genetic diagnosis exemplifies the future promise of CB transplantation [9]. Several studies over the past decade have examined the use of CB from a related donor for transplantation. Summary analyses of these individual studies have been done by the International Cord Blood Transplant Registry [7,10] and the Eurocord Registry [5,11] (Table 2).

CB Transplantation From an Unrelated Donor

The use of CB from an unrelated donor is thought to have several advantages: (1) it is more accessible than marrow because of reduced HLA restriction; (2) a shorter interval is required between the search for a donor and acquiring CB; (3) it poses no risk to the donor; and (4) it may involve a lower risk of acute GVHD [3-5,12-14]. Clinical studies on CB from unrelated donors suggest that these advantages are real.

Placental Blood Program at the New York Blood Center. This study, composed of 98 transplantation centers worldwide, provides the largest dataset of CB transplantation from unrelated donors (Table 3). As of 1998, cumulative rates of engraftment based on actuarial analysis for 562 patients were 81% for neutrophil recovery (median of 28 days) and 85% for platelet engraftment (median of 90 days). Several factors were associated with events related to the transplantation, including degree of HLA disparity, underlying disease, patient age, number of leukocytes in the graft, and the transplantation center. Leukocytic content of the graft was associated with the length of time to engraftment, but it was not significantly related to event-free survival after engraftment (unlike age, which was significantly associated with event-free survival). This suggests that larger doses of leukocytes may speed up engraftment but may not improve event-free survival, particularly for older patients [6].

Eurocord Registry. As of April 1998, 158 children and adults had received CB from unrelated donors through the Eurocord Registry (composed of 39 transplantation centers from 15 countries) (Table 3) [5,15,16]. Separate overall analyses were done for 102 children and 44 adults, as well as a subset analysis of 42 adults with malignant disease.

Children. The report of 102 children who received unrelated CB showed an overall 1-year survival rate of 37%; neutrophil engraftment in 74% of patients; incidence of acute GVHD ≥II in 38% of patients; and overall survival rates of 35% in patients with malignancies, 10% in patients with bone marrow failure syndromes, and 70% in patients with inborn errors. Factors favorably associated with survival were ABO match (P = .01) and cytomegalovirus (CMV)-negative status before CB transplantation (P = .02). No significance was found between survival and weight, age, number of nucleated cells infused, sex, or HLA disparity. No association was found between GVHD and the number of HLA mismatches. (For the 44 adults listed in this registry during the same time period, the overall survival rate was 16%.)

Adults With Malignancies. When only the 42 adults with malignancies were analyzed, overall survival rate at 1 year was 17%. Factors favorably associated with survival were cell dose (≥1 × 10^7/kg) (P = .0001) and having a good risk status at the time of transplantation (P = .02). The number of infused nucleated cells ranged from 0.2 to 6 × 10^7/kg (median, 1.7 × 10^7/kg). The 6 patients who received <1 × 10^7/kg died. The 1-year survival rate of poor-risk patients versus good-risk patients was 7% versus 36%. Median time to neutrophil recovery (≥500/mm^3) was 35 days; median time to platelet engraftment (≥20,000/mm^3) was 176 days. Acute GVHD (>grade II), observed in 18 patients, was not associated with HLA disparities (P = .58) [15].

These studies indicate that CB transplantation benefits children with malignant disease, deficient immune systems, or inborn errors, whereas it may be less beneficial in adults and in patients with bone marrow failure syndromes, especially poor-risk adults (Table 3).

Table 2. *Studies of Cord Blood Transplantation From Related (Sibling) Donors*

	International Cord Blood Transplantation Registry [10]	Eurocord Registry [5,11]
Number of patients	**62**	**102**
Median age, y (range)	**(0.5-16)**	**5 (0.2-20)**
Median body weight, kg (range)	**—**	**19 (5-50)**
Diseases, n	**—**	**Malignancies (61)**
	—	**Nonmalignancies (41)**
Number of HLA mismatches, n	**0 (51)**	**0 (80)**
	1-3 (11)	**1 (5)**
		2 (6)
		3 (10)
		4 (10)
Overall survival at 1 year	**—**	**64%**
Survival by HLA status	**61% at 2 y (0-1 HLA disparity)**	**73% at 1 y (0 HLA disparity)**
		50% at 1 y (1-4 HLA disparity) (*P* = .006)
Survival at 1 year by disease	**—**	**55% (malignancies)**
		67% (bone marrow failure syndrome)
		100% (hemoglobinopathies)
		71% at 1 y (inborn errors)
Incidence of GVHD	**6/62 (grades 0-1)**	**24% (grade >II)**
	3/62 (grades II-IV)	**7% (grades II-IV)**
	3/62 (chronic)	**3 of 43 patients at risk (chronic)**
Neutrophil engraftment, d (median)*	**9-46 (22)**	**8-49 (28)**
Platelet engraftment, d (median)†	**15-117 (51)**	**4-180 (48)**

*Neutrophil recovery defined as time to achieve an absolute neutrophil count >5×10^8/L.

†Platelet count >5×10^{10}/L untransfused for 7 days.

Duke University and University of Minnesota Study

The University of Minnesota and Duke University have collaborated on the study of unrelated donor CB transplantation for 7 years. A subset of 257 patients was selected for analysis based on the following criteria: (1) patients had to have received a transplantation at least 100 days prior to analysis; (2) patients had to have received cytoreductive therapy prior to CB transfusion; and (3) donor and recipient pairs had to have HLA disparity of 0 to 3 antigens. Of these patients, 164 (64%) had malignant diseases (most with acute leukemia) and 93 (36%) had nonmalignant diseases. Most patients received a 2-mm HLA mismatch (48%). All patients received prophylactic treatment for GVHD, with the majority receiving cyclosporin A plus low-dose methylprednisolone (72%). Prophylactic granulocyte colony–stimulating factor (G-CSF) was given to 153 patients, either 5 μg/kg (17%) or 10 μg/kg (74%) (Wagner, presentation at CME symposium, San Francisco, California, December 1, 2000).

Results from this subset study show the following:

- Survival. The overall survival rate was 50% at 1 year and 41% at 3 years. Analysis by risk factors showed a better survival for (1) younger patients (similar to BMT), (2) patients treated with more than 1.5×10^7/kg cell dose, (3) patients who received $CD34^+$ cell dose of more than 1.5×10^5/kg, and (4) patients who were Caucasian (similar to BMT).
- Engraftment. Neutrophil engraftment was achieved in 87% of patients at a median of 25 days. Platelet recovery was achieved in 51% of patients at a median of 6 months. Of all the demographic, graft, and treatment risk factors analyzed, nucleated cell dose ($\times 10^7$) had the most effect on engraftment. Cell doses lower than 1.5×10^7 show a delay in recovery and an inferior engraftment. These data suggest that 1.5×10^7 should be the lowest acceptable graft dose.
- GVHD. Only 12% of patients experienced acute GVHD (grades III-IV), a rate that is about half of what was expected even in a pediatric population receiving transplants of bone marrow. Analysis by risk factors did not show any association between acute GVHD and HLA match or age. The most dramatic difference with CB was found for chronic GVHD, which occurred in only 7% of patients with no difference between adult and pediatric populations. Analysis by risk factors showed a trend toward higher incidence of GVHD in patients treated with melphalan.

Overall, this study showed that CB transplantation from an unrelated donor was associated with (1) a high rate of engraftment in recipients of $\geq 1.5 \times 10^5$ $CD43^+$ cells/kg recipient weight, (2) a delay in platelet engraftment of 6 months (median) that was dependent on cell dose, (3) a delayed neutrophil recovery of 25 days (median) that was dependent on cell dose, (4) a low rate of acute and chronic GVHD even with a high degree of HLA disparity, and (5) high risk of regimen-related mortality in recipients of low cell doses. Based on the clinical experience at Duke University and University of Minnesota, the cell dose of 1.5×10^7

Table 3. *Studies of Cord Blood Transplantation From Unrelated Donors*

	New York Blood Center [6]	Eurocord Registry [5,15,16]	Eurocord Registry [5,15,16]	University of Minnesota/ Duke University*
Number of patients	562	158†	42‡	257
Age, y (n)	<2 (114) 2-5 (127) 6-11 (137) 12-17 (82) ≥18 (102)	Children: 0.2-14 (102) Adults: (44)	15-50 (median, 26)	0.2-58 (median, 8.1)
Body weight, kg (n)	<10 (77) 10-19 (148) 20-39 (152) 40-59 (91) ≥60 (94)	5-46 (102)	35-90 (median, 56)	3.9-102.8 (median, 24.5)
Diseases (n)	Leukemia or lymphoma (378) Genetic disease (137) Acquired disease (47)	Leukemia or lymphoma (72 children) Bone marrow failure syndrome (12 children) Inborn errors (18 children) Malignancies (42 adults)	Acute leukemia (16) Chronic myeloid leukemia (21) Non-Hodgkin's lymphoma (2) Myelodysplastic syndromes (3)	Malignancies (164) Nonmalignancies (93)
Number of HLA mismatches (n)	0 (40) 1 (218) 2 (261) 3 (37) 4 (3)	Children: 0 (14) 1 (64) 2 (23) 3 (1) Adults: 0 (6) 1 (17) 2 (14) 3 (5)	0 (2) 1 (8) 2 (21) 3 (10) 4 (1)	0 (18) 1 (91) 2 (124) 3 (15) Unresolved (9)
Platelets engraftment (median time)	85% at 180 d (90 d)	—	—	51% (6 mo)
Neutrophil engraftment (median time)	81% at 42 d (28 d)	74% for children	76 ± 12 at 60 d	87% (25 d)
Incidence of GVHD (grade)	23% acute (III-IV) 25% chronic	38% acute (≥II)	18 acute (≥II) 3 chronic	30% acute (≥II) 12% acute (III-IV) 7% chronic
Survival rate (disease)		Children: 37% (overall) 35% (malignancies) 10% (bone marrow failure syndrome) 70% (inborn errors) Adults: 16% (overall)	17% ± 6% (overall)	41% at 3 years

*Data presented at CME symposium.

†Patients analyzed were divided into 2 groups: children (n = 102) and adults (n = 44).

‡Separate analysis of 42 adult patients with malignancies.

nucleated cells per kilogram patient weight would appear to be the threshold dose for safety of CB transplantations.

National Heart, Lung, and Blood Institute Study

A prospective safety and efficacy study of unrelated donors for CB transplantation initiated in 1998 by the National Heart, Lung, and Blood Institute is designed to further test whether CB provides an adequate source of hematopoietic progenitor cells for all or only a subgroup of patients. The primary end point is 180-day survival, with everyday end points of disease-free survival, engraftment, incidence and severity of GVHD, relapse, infection, and immune reconstitution. Patients will be stratified on the basis of HLA disparity: 6/6 and 5/6 versus 4/6 versus 3/6 and <3/6 [17]. This study is presently underway and no results are yet available.

General Conclusions From Studies: Places for Further Research

The results of these studies point to several areas in need of further research.

Nucleated Cell Dose. The threshold dose of nucleated cells that is adequate for successful engraftment for adults and children must be established. Most data show a significant decrease in survival in adult patients infused with $\leq 1.5 \times 10^7$/kg nucleated cells. The New York Blood Bank, Eurocord, and the combined University of Minnesota and Duke University studies all indicate a strong dose relationship between nucleated cell dose and engraftment.

Disease Stage and Engraftment. The influence of disease state on delayed or successful engraftment in patients with specific diseases should be determined. The New York

Blood Center found that certain diseases, such as chronic myelogenous leukemia and aplastic anemia, were more likely to have graft failure. In contrast, the combined study from the University of Minnesota and Duke University found only a relationship between aplastic anemia and Fanconi's anemia and graft failure. However, these latter diseases are also associated with graft failure after unrelated donor BMT.

HLA Status and GVHD. The influence of HLA status on the incidence of GVHD must be established. This statement is supported by a recent study of HLA-identical siblings that found a lower incidence of acute and chronic GVHD in CB recipients compared with bone marrow recipients from HLA-identical siblings [7]. The studies summarized here do not show a relationship between HLA disparity and GVHD, and the rates of GVHD seem lower than expected from historical experience.

This encouraging clinical experience increases the importance of basic research into the biological and immunological properties of CB to improve the effectiveness of CB transplantation [10,12].

BIOLOGICAL RESEARCH: EXPANSION AND TRANSDUCTION OF CB CELLS

Because successful engraftment depends on an adequate dose of hematopoietic stem cells (HSCs), CB is currently limited as a graft for adult transplant recipients. In addition, increasing cell dose may shorten the time to engraftment and thus improve the success of the therapy. Finding ways to expand the dose of HSCs in CB is therefore one of the primary goals in current CB transplantation research. Expansion of these cells is based on the theory that the unique properties of HSCs, which include the capacity of long-term marrow reconstitution and the ability to generate large numbers of committed progenitors with progressively restricted differentiation potential, should be retained after these cells are transplanted in the clinical setting. A number of protocols have been designed for the ex vivo expansion of CB HSCs, a sampling of which are discussed below. These studies also examine whether cultures that support CB HSC expansion allow retroviral transduction and whether engrafted cells in culture possess self-renewal activity.

CB Expansion Studies

Adult marrow contains a greater number of $CD34^+$ cells than does CB (ie, frequencies of $CD34^+$ cells are about .01% to 1.0% nucleated cells in CB and about 1% to 3% nucleated cells in adult bone marrow).

However, CB has a higher in vitro proliferation or expansion potential than does adult bone marrow, as illustrated by in vivo studies of NOD/SCID mice [18]. Because of the capacity of CB HSC to expand, several investigators are attempting to expand these cells ex vivo with the hope of eventually making these cells available for clinical use in the transplantation setting [19,20]. Studies in laboratory mice show that early and late engraftments in mice depend on stem cell dose, and mice that received transplants of only committed progenitors had graft failure. In humans, supplementation of committed progenitors results in earlier engraftment. Therefore, early and long-term engraftments require both expansion of committed progenitors and stem cells.

Most studies on CB expansion, until recently, have used the antigen $CD34^+$ and its subsets to detect the presence of HSCs in vitro. The immunophenotype $CD34^+CD38^-$ is thought to define a subgroup of primitive progenitor cells that have a higher cloning efficiency in CB than in adult bone marrow. Cells with this immunophenotype in CB also expand more rapidly when stimulated by cytokines interleukin (IL)-3, IL-6, and stem cell factor (SCF). [8]. More recent evidence suggests that there are some HSCs that do not express CD34 antigens [21-23], and therefore investigators are using a variety of culture conditions and assays to expand the HSCs in CB.

University of Minnesota Experience

Culture Conditions for CB Expansion. The AFT04 culture system, in which immortalized myofibroblasts (MSCs) derived from mouse fetal liver are used to support MSC, has been shown to (1) support repopulating murine stem cells for more than 6 weeks in vitro [24] and (2) support human CB, bone marrow, peripheral blood long-term culture initiating cell (LTC-IC), and extended LTC-IC in both "contact" and "noncontact" cultures. This culture system (transwells above the AFT024 feeder) was used in a number of experiments to evaluate expansion and transduction of CB HSCs. The in vivo assays of SCID repopulation were used to measure CB HSC in CB cells that were transplanted in NOD-SCID mice [25] and preimmune fetal sheep [26]. The in vitro assay used to measure primitive progenitors that could undergo self-renewal and differentiation to lymphoid and myeloid lineages was the myeloid-lymphoid initiating cell (ML-IC) assay [27].

Verfaillie and colleagues showed that AFT024 noncontact cultures of $CD34^+$ $CD38^-Lin^-$ cells using different combinations of cytokines (FS7 = F1t3-L, SCF, IL7; FS7T = F1t3-L, SCF, IL7, thrombopoietin [Tpo]; FT = Flt3-L, Tpo) were able to induce expansion of committed and primitive progenitor cells. At 5 weeks, there was a 15-fold expansion of $CD34^+Lin^-$ CB colony-forming cells (CFC), a 5-fold expansion of LTC-ICs, and a 10-fold expansion of natural killer-initiating cells (NK-ICs) [27]. In addition, they showed that the AFT024 noncontact culture supports maintenance of ML-IC. These studies show that very primitive progenitor cells are maintained in the culture system and suggest that stem cells may also be preserved in these cultures even though they have not yet been able to be expanded. Using this same noncontact culture showed that CB ML-ICs were able to undergo self-renewing cell divisions in vitro [27].

Cells capable of engrafting NOD-SCID mice are maintained as demonstrated in the limiting dilution assay showing that the number of SCID repopulating cells (SRCs) that engraft in primary and secondary animals is similar in unmanipulated cells and cultured cells at 7 days or 14 days. No engraftment has been seen in tertiary animals with unmanipulated or manipulated cells. When transplanted in fetal sheep, no difference in engraftment was found between unmanipulated cells and cultured cells at 7 days. These experiments show that self-renewing cells and committed progenitor cells can be maintained ex vivo. This culture system also supports the transduction of 50% to 80% CB LTC-IC, ML-IC, and SRCs with an GALV-pseudotyped eGFP retrovirus.

Because of growing evidence that the CD34 antigen is not present on all HSCs [21-23], Verfaillie and colleagues tested whether the AFT024 feeder culture system would support ML-IC in CB $CD34^-Lin^-CD38^-$ cells. They found that cultures with FT (Flt3-L and Tpo) generate $CD34^+Lin^-$ cells with ML-IC ability. This finding shows that the AFT024 feeder culture system supports both $CD34^+$ and $CD34^-$ CB HSCs.

To test for a more clinically suitable culture system (because noncontact cultures cannot be used in the clinic), Verfaillie and colleagues used cultures with media conditioned by the AFT024 feeder or with "artificial conditioned medium" with cytokines and extracellular matrix components secreted by the AFT024 feeder to test whether similar results could be obtained. They found that in "artificial conditioned medium" cultures, cells capable of repopulating NOD-SCID mice can be maintained to the same extent and that these cultures can support expansion of more committed CFC and LTC-IC that is equal to or greater than expansion in AFT024 noncontact cultures [28,29].

Retroviral Transduction of Expanded Progenitors. Because similar expansion of progenitors and similar preservation of SRCs were found in stroma-free systems compared to AFT024 noncontact systems, it is also important to determine whether similar transduction can be obtained in these culture systems. In AFT024 noncontact culture systems, 60% to 80% (mean, 74%) of CB $CD34^+Lin^-$ ML-ICs are transduced and >80% of CB $CD34^+Lin^-$ SRCs are transduced. However, despite expansion of CB CFC, CB LTC-IC, and CB NK-IC in a stroma-free system, there is poor transduction of these progenitor cells. They found that the dose of GAGs used in these cultures is an important determinant for successful transduction. High doses of 10 μg/mL prevented transduction of progenitors, whereas lower doses of 5 μg/mL allowed for transduction. Other studies have shown successful engraftment of NOD-SCID mice with retrovirally transduced CB [30-35].

Summary. As a result of this work, culture methods are available that allow both progenitor expansion and transduction and that are suitable for the clinical setting. In the laboratory setting, AFT024 noncontact culture supplemented with FT (Flt3-L, Tpo) supports the following:

- expansion of committed CB $CD34^+$ progenitors and maintenance (and possibly expansion) of primitive CB $CD34^+$ ML-IC and SRC;
- transduction of CB $CD34^+$ committed and primitive progenitors with murine retrovirus based vectors; and
- generation of $CD34^+$ ML-IC from the quiescent $CD34^+$ stem cell pool.

Stroma-free cultures, based on factors present in AFT024 conditioned medium, (1) support expansion of committed CB $CD34^+$ progenitors and maintenance (with the possibility of expansion) of primitive CB $CD34^+$ ML-IC and SRC; and (2) can prevent transduction of progenitors if the dose of GAGs added to the artificially conditioned medium is too high.

Two clinical trials now underway are testing the time to and durability of engraftment and the contribution of expanded graft to early and late engraftment by either restriction fragment length polymorphism (RFLP) or retroviral marking.

University of Torino Experience

Culture Conditions for CB expansion. A miniwell expansion system using stroma-free cultures of $CD34^+$ cells was used to expand HSC in the laboratory setting. This laboratory system showed that a degree of stem cell self-renewal could be triggered under certain culture conditions. This system used a number of assays for in vitro detection of committed progenitors (semisolid cultures, colony-forming units–granulocyte/macrophage [CFU-GM], burst-forming unit-erythroid [BFU-E], CFU-megakaryocyte); more primitive progenitors (multipotent colony-forming units [CFU-GEMM], CFU-B1, high proliferative potential [HPP]-CFC, PRE-CFC); and surrogate in vitro assays for "putative stem cells" (long-term cultures: LTC-IC, at 5, 8, and 12 weeks). CB $CD34^+$ cells can be maintained in culture for more than 7 months in a stroma-free, serum-containing system in the presence of the cytokines Flk2/Flt3 ligand (FL) and Tpo, with or without c-kit ligand (or SCF). In these cultures, CB $CD34^+$ can undergo massive expansion: 20-million-fold for progenitors and 270,000-fold for more primitive LTC-ICs [18,36].

Engraftment Capacity in NOD/SCID. Using an in vivo transplantation model in irradiated SCID and NOD/SCID mice, CB NOD/SCID long-term in vivo repopulating $CD34^+$ cells could be expanded 70-fold after 9 to 10 weeks in cultures that included FL, TPO, and SCF with or without IL-6 [37]. Fetal calf serum can be replaced by human serum without impairing the ability of the expanded cells to engraft to the sublethally irradiated recipients [37].

There is a low level of engraftment in mice receiving transplants of low amounts of purified $CD34^+$ CB cells (2×10^4), but increasing levels of engraftment with increased amounts of $CD34^+$ cells and in the manipulated (expanded) cells. This indicates that inoculation with accessory $CD34^-$ cells and with $CD34^{+++}$ cells has no effect on the degree of engraftment, and therefore accessory cells do not play a role in the increased ability of expanded $CD34^{+++}$ cells to repopulate.

Self-Renewal Ability of Engrafted Cells. Human CB $CD34^+$ cells expanded for 4 or 6 weeks were able to completely and durably repopulate the bone marrow of a myeloablated NOD/SCID mouse, and human $CD34^+$ cells retrieved from the engrafted bone marrow could repopulate the marrow of a secondary irradiated recipient.

Summary. These studies have shown the following:

- Accessory cells do not affect the increased ability of expanded $CD34^{+++}$ cells to repopulate.
- Engrafted human cells retain their self-renewal capacity.
- Characterization of culture conditions that allow human hematopoietic stem cell expansion is an important requirement for the successful implementation of many clinical transplantation and gene therapy protocols.
- These studies represent a significant step toward the practical realization of such approaches.

IMMUNOLOGICAL RESEARCH: PROPERTIES OF CB

The recent publication of a large study [7] that confirms earlier preclinical and clinical studies that suggested a lower incidence of GVHD in CB stem and progenitor cell transplantation [38-40] raises several issues about the immuno-

Table 4. *Considerations in Establishing a Cord Blood Bank*

Specific Issues	Unresolved Issues
Donor Recruitment	Adequate cell dose
Consent	Speed of engraftment
Donor Suitability	Histocompatibility requirement
Collection	Expansion potential
Stem cell selection or red cell depletion	Optimum processing procedures
Preservation	Duration of storage
Histocompatibility testing	Autologous use potential
Genetic diseases testing	Role of maternal cells in CB
Transplant specimens	Role of genetic testing for disease
Transportation	Approaches for testing opportunistic diseases
Thawing and transfusion	
Confidentiality	

logic properties of CB. This section summarizes recent studies that show differences between CB immune cells and immune recovery after CB transplantation.

Differences in Immune Cells Between CB and Adult Blood/Bone Marrow

There have been a number of reports in which a comparison between immune cell types and function of CB cells with adult blood or bone marrow cells have suggested that CB immune cells may be more immature and less functionally active than their adult counterparts [40-43]. For example, CB T cells manifest less cytotoxic activity than adult T cells after primary, secondary, and tertiary allogeneic cell stimulation [44,45]. Moreover, whereas CB T cells respond as well as adult T cells to the proliferation-inducing activity of a primary allogeneic stimulation, CB T cells, in contrast to adult T cells, become unresponsive to secondary allogeneic stimulation. Adult T cells proliferate to an even a greater extent after secondary compared to primary allogeneic stimulation [46]. The mechanisms of this tolerance of CB T cells to secondary allogeneic cell stimulation reflect the intracellular status of the CB T cells in that the inactive guanosine diphosphate (GDP)-bound form of Ras is not activated to the active guanosine triphosphate (GTP) form [47]. More recent studies note that human CB has few or no cells with a $CD8^+$ natural killer (NK) T-cell phenotype. However, IL-12 and IL-15 were found crucial to induction of $CD8^+$ NKT cell development [48]. This development was associated with expression of the co-stimulating receptor 41BB. Because NKT cells are potent cytotoxic cells, it is possible that lack of these cells in CB may account, in at least part, for the previously noted low allogeneic cytotoxicity by CB T cells.

Recovery of Immune System After CB Transplantation

The relative immaturity of some immune cells in CB presents a potential problem for recovery of immune cells after CB transplantation. A study from Indiana School of Medicine was conducted in part to evaluate immune recovery in patients receiving CB from an unrelated donor [49]. Twenty-seven patients (14 male and 13 female), with a median age of 4.85 years (range, 0.4-17.1 years) and a median weight of 18.4 kg (range, 5.65-71.4 kg), received a total of 30 transplants between November 1994 and February 1999. For immune parameters, T-cell recovery was 9 to 12 months, B-cell recovery was 6 months, and NK-cell recovery was 2 months. For cell function recovery, T-cell recovery was 6 to 9 months, B-cell recovery (immunoglobins) remained in the normal range with nadirs between 1 and 3 months, and NK-cell recovery was 1 month in all children.

Immune Recovery and Cell Dose. The study by Thomson and colleagues [49] found (1) no relationship between the rate of T-, B-, and NK-cell numerical recovery compared to nucleated cells/kg infused and CD34 cells/kg infused, (2) only a weak association between phytohemagglutinin (PHA) recovery and nucleated cells/kg infused, (3) no relationship between the rate of T-, B-, and NK-cell numerical recovery and degree of HLA mismatch, (4) only a weak relationship between CD8 cell recovery and grade ≥II GVHD, (5) a total of 13 grade II and 3 grade IV infections in the first 100 days after transplantation, and (6) no correlation between grade of infection and time to immune recovery in this series (which at 27 is small).

Summary. These studies summarize some knowledge on the immunological properties of CB and areas that need further research:

- Lower numbers of $CD8^+$ NKT cells in CB compared to adult peripheral blood may account for the weak cytotoxicity of T cells in CB.
- Low or absent $CD8^+$ NKT cells in CB may be involved in the lowered GVHD noted after CB transplantation.
- Studies are needed to test $CD8^+$ NKT cells after CB transplantation, considering that induction of CB $CD8^+$ NKT cells may be associated with antitumor activity.

CB BANKING

With the increased recognition that CB is a viable source of hematopoietic cells for transplantation, the need for making available large numbers of high-quality CB units has led to the creation of CB banks worldwide. CB banking has several potential benefits: (1) rapid availability of CB, (2) no donor risk or attrition, (3) low risk of transmitting infectious diseases, (4) potentially reduced risk of acute GVHD, and (5) possible increased ability to expand the pool of donors to include ethnic and racial minorities [10].

The first operational CB banks were established in 1993 in New York, Milan, and Düsseldorf. Other CB banks have since followed worldwide. Although as of November 2000, Bone Marrow Donors Worldwide (BMDW) estimated the current worldwide inventory of CB as 54,157 units, it is estimated that this number probably exceeds 70,000 units. This number is the sum of the 54,157 units listed by BMDW; the 14,000 units stored in 20 banks located in Japan [9], China [6], Korea [3], Thailand [1], and Singapore [1] (T. Takahashi, written communication); and the approximately 3,000 units present in some banking programs not yet listed by the BMDW. The establishment of these banks, and the subsequent development of coalitions of CB banks and organizations devoted to establishing quality, require addressing several issues, some of which have been successfully addressed and others that remain debated.

Table 5. *Selected Organizations That Ensure Quality and Standards in Cord Blood Banking**

American Association of Blood Banks (AABB)
American Red Cross (ARC)
Bone Marrow Donors World (BMDW)
Cord Blood Transplantation Study (COBLT)
European Blood and Marrow Transplant Group (EBMT)
Eurocord
Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT)
Group for the Collection and Expansion of Hematopoietic Cells (GRACE)
International Society for Hematotherapy and Graft Engineering (ISHAGE)
Joint Accreditation Committee of ISHAGE-Europe and EBMT (JACIE)
NETCORD
National Bone Marrow Donor Program (NMDP)

*From Rebulla, presentation at CME symposium.

Major Issues of CB Banking

Many of the major issues involved with the establishment of a CB bank are similar to the issues involved with the establishment of traditional blood banks. Techniques to efficiently and safely select donors and to collect, store, process, and distribute CB units to areas of need throughout the world have been developed, with ongoing assessment of ways to improve these techniques [50-52]. Other issues remain unresolved, with studies underway to address them (Table 4). Among these issues are the biological and immunological unknowns of CB that basic science researchers are studying, such as adequate cell dose, speed of engraftment, and expansion potential. Other areas involve the unknowns of long-term storage. A recent examination of defrosted CB samples that were processed, stored, and frozen for up to 15 years at Indiana University School of Medicine suggests that long-term durability of cryopreserved CB cells is viable given the high, efficient recovery these stem and progenitor cells maintained, along with extensive capacity for proliferation and differentiation (Broxmeyer, presentation at the CME symposium).

All of these issues are vitally important to the establishment of high-quality CB banks. Quality of CB banking refers to both the quality of the product (CB) and the quality of the systems used to make CB available. In recognition that development of CB banks depends on establishing product standards and quality systems, several organizations have been formed to ensure the highest standard of CB collection, exchange, and transportation nationally and internationally (Table 5).

CB Banking: American Red Cross

As of November 2000, 38% (19,948 CB units) of the BMDW file was contributed by 6 banking programs in the United States, one of which is the American Red Cross. The American Red Cross Cord Blood Program was established in 1998 and has focused on the following goals.

Creating Multiple Sites. Four regional sites have been created to collect and process CB nationwide. Currently, a total of 2000 HLA units have been collected and processed at the 4 sites. All of these CB units are from allogenic donors. Each site, representing a different national geographical and ethnic community, is pursuing specific research goals:

1. The Columbus Program (Ross Cord Blood Program) was the first program developed by the American Red Cross. CB is collected by trained staff of the American Red Cross. The program's primary interest is in ex vivo expansion and improved processing methods.
2. The Western Area Program includes collection and processing sites in San Diego, California, and Portland, Oregon, and is the fastest-growing program. The goal of this program is to collect 30,000 blood cord units from ethnically diverse populations over the next 5 years. Current blood collection shows that 57% of the donors are white, 25% Hispanic (making this site the largest collection of CB from Hispanic donors), and 18% are from other ethnic groups. Similar to the Columbus site, CB is collected by American Red Cross trained staff.
3. The North Central Area Program includes collection and processing sites in Minneapolis and St. Paul, Minnesota. This program is specifically studying the consent process (including paternal consent) and techniques for the short-term liquid preservation of CB stem cells.
4. The New England Region Program began in 1997 at the University of Massachusetts and was acquired by the American Red Cross in 1999. CB is collected by trained obstetrical staff using the technique of the placenta in utero. This program is studying donor characteristics and Phase I clinical trials.

Developing Common Investigational New Drug Applications and Procedures. A main goal of the American Red Cross is to establish common procedures and standards. Central to this goal is the need to address a number of ethical issues.

Increasing Minority Recruitment. Because the majority of CB units currently available are disproportionately from Caucasian donors, there is a strong need to increase donor participation by other ethnic groups. Along with developing multiple collection and processing sites nationwide to include the many diverse ethnic groups in the United States, the American Red Cross also is involved with studies specifically targeting minority recruitment.

A multicenter study through the National Marrow Donor Program and 5 CB Banks (Worchester, New York, Denver, San Diego, and Gainesville) and the corresponding bone marrow donors in each geographical location was initiated in 1998 to compare minority recruitment of CB donors with minority recruitment of bone marrow donors. Two end points were studied: (1) the race of CB donors compared with the race of all women who delivered babies at participating hospitals, and (2) the race of bone marrow donors compared with race of geographical area based on Census data. A limitation to the study was the way in which the racial makeup of the donors was obtained, which was simply through completing a self-reported questionnaire. The results of the study showed that (1) the number of recruited CB units from minority donors was always less than the number of minority women giving birth; and (2) none of the CB banks recruits a higher percentage of minorities compared to the baseline delivery population than do the bone marrow donor centers compared to the baseline Census

Table 6. *European Cord Blood Banking Programs*

Austria
Belgium
Bruxelles (Leuven)
Czekia
Düsseldorf
Finland
France (Paris, Besancon, Bordeaux)
Germany (Ulm)
Italy (GRACE: Florence, Milan, Padua, Rome, Turin)
Netherlands
Spain (Barcelona, Malaga, Galicia, Madrid, Valencia, Canarias)
Switzerland
London
Warsaw

population. Overall, the study found no advantage of CB compared to bone marrow in terms of minority recruitment, which indicates a need for improved strategies to increase minority donor participation.

Participating in CB Banking, Research, and Clinical Investigation. A number of research projects are underway by the American Red Cross, including studies on cytokine expansion, engraftment into immunodeficient mice, donor selection, minority recruitment, consent process, and process and collection techniques. Three major studies are currently underway for the year 2001: (1) CB donor evaluation (discussed below), (2) optimization of HPC recovery from CB during processing, and (3) expansion of stored CB cells.

The Cord Blood Donor Evaluation study included more than 1200 donors from the New England Program site and compared donor (donor age, race, smoking history) and baby (birth order, weight, and sex) characteristics to laboratory parameters of the processed CB units (volume, nucleated cell counts, CD34 counts, and CFU-GM). Results of the study showed that (1) large volume units correlated with higher nucleated cell counts, CD34, and CFU-GM; (2) no effect was found between maternal age or race on any of the lab parameters; (3) smokers had lower CD34 counts; and (4) first babies, as well as bigger babies, had higher nucleated cell counts, CD34, and CFU-GM. A multivariate analysis of the effect of smoking, number of previous births, gestation duration, and birth weight on total nucleated cell count, CD34 count, and CFU-GM found that birth weight had the largest effect on all counts. For every 500-g increase in weight, there was a corresponding 10% increase in total nucleated cell count, 28% increase in CD34 count, and 21% increase in CFU-GM. The results indicate that (1) bigger babies and first babies may be better CB donors, (2) data can be used to select the best CB donors and to preserve resources, and (3) banks can expand and provide more available CB units by optimal use of resources.

CB Banking in Europe

As of November 2000, 54% of the BMDW file was contributed by European banks (29,152 CB units). Analysis of the Eurocord database (E. Gluckman and V. Rocha, Paris, written communication) and of the existing published reports indicates that about 20% of CB transplantations so far performed worldwide were provided by European banks (Table 6). Soon after the establishment of the first CB banks in Europe (Milan CB Bank and the Düsseldorf CB Bank), a major concern was the importance of helping physicians select high-quality CB. From this concern evolved the idea of combining the resources of CB banks to ensure quality.

Group for the Collection and Expansion of Hematopoietic Cells. The first attempt at combining resources was initiated by the Milan Cord Blood Bank in 1995. The Group for the Collection and Expansion of Hematopoietic Cells, called GRACE, was created to facilitate communication among clinicians and investigators in Italy [53]. This national model linked banks to a central hub in Milan. Seven banks became members of this organization, 5 of which (Milan, Turin, Florence, Rome, Padua) had International Organization for Standardization (ISO) 9002 status. ISO 9002 is a model for quality assurance developed in 1987 and updated in 1994; it includes 20 clauses on procedures, organizational structure, processes, and resources for CB banking. A bank with a ISO 9002 status is certified as compliant with the specific clauses denoting quality in CB banking [54]. Several protocols were formally approved by GRACE, including (1) CB collection, characterization, and cyropreservation; (2) CB unit data transmission; (3) CB unit extended search, shipment, and patient follow-up; and (4) clinical protocol for related and unrelated CB transplantation. The success of this program encouraged the possibility of exporting it internationally to provide clinicians access to CB information, to create harmonization among CB banks, and to ensure quality assurance on a worldwide level.

Netcord. In March 1997, a pilot program called Netcord was initiated to fulfill this goal. It was a combined effort between the Milan and Düsseldorf CB banks to share inventories of CB by using a software program to perform searches of shared inventory. In 1997-1998, the number of duplicate searches dropped significantly, only 16% of 2,953 searches. In 1998, CB banks in London and Barcelona joined the group. From 1998, this group evolved into a consolidated international network of banks linked to 2 hubs (1 in Milan and 1 in Düsseldorf) that could search the whole inventory. This group operates on standards that were published in June 2000 by Netcord and the Foundation for the Accreditation of Hematopoietic Cell Therapy in a publication called The International Standards and Inspection Checklist for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release (first edition). As of November 2000, 23,835 units were available and 235 units transplanted (1995-2000) from the 2 hubs that link a number of participating CB banks in Düsseldorf, Milan, New York, Barcelona, Denver, Leiden, London, Paris, St. Louis, and Tokyo. The program continues to evolve, with many other countries applying for participation. Several systems in place help with the movement of information: the Eurocord Registry does patient follow-up, and the recent creation of a virtual office now allows searches on the Internet.

CONCLUSION

CB transplantation has expanded the ability of the transplantation community to meet the growing needs of their patients. Clinical data over the last decade show promising results in CB transplantation using blood from related as

well as unrelated donors. Basic science continues to look for ways to expand the quality and quantity of CB. CB banks are now established around the world, and major efforts are underway to standardize banking to facilitate regulation, collection, processing, and distribution as a way of providing the highest-quality CB for patient use.

ACKNOWLEDGMENT

The authors express their appreciation to Mary Beth Nierengarten, MA, ELS, for assistance in preparing this report.

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