# Umbilical Cord Blood Transplantation

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**ABSTRACT**

The number of umbilical cord blood transplantation is increasing worldwide as it has expanded the ability of the transplantaion community to meet the growing needs of their patients. Clinical data over the last decade show promising results in transplantation using both related as well as unrelated cord bloods. Cord blood banks are essential for the clinical use for transplantation and are now established around the world with the major efforts to standardize banking in collection, processing and distribution of cord blood for providing the highest quality stem cells for the patients. In Korea, Medipost, Histostem and some regional cord blood banks were established some years ago and collected thousands of cord blood for public but it had some limitations and was not expanded as the cord blood transplantation was not covered by medical insurance. Recently with the change in the policy of medical insurance to cover the cord blood transplantation, several venture companies are showing great interests in cord blood banking and trying to establish private cord blood banks in Korea. This review article discusses the current status of cord blood transplantaion and also the clincial use of stem cells from cord blood. **(Immune Network 2003;3(2):83-88)**

**Key Words:** Umbilical cord blood, transplantation, stem cell

## Introduction

Since the first successful transplantation using umbilical cord blood (UCB) to treat a patient with Fanconi's anemia in 1988 (1), cord blood transplantation (CBT) has become an alternative to bone marrow transplantation (BMT) to treat a varitey of diseases. The UCB donor was his human leukocyte antigen (HLA) identical sister and fifteen years later, he is doing well with full donor hematopoietic and lymphoid reconstitution. Dr. Gluckman is proudly presenting his pictures in every cord blood (CB) meeting showing his normal growth. The first success opened the way to an entire new field in the domain of allogeneic hematopoietic stem cell (HSC) transplantation as it showed that a single UCB unit contained sufficient numbers of HSCs to reconstitute definitively the host lympho-hematopoietic compartment and an UCB unit could be collected at birth without any harm to the newborn infant and UCB HSCs could be cryopreserved, thawed without losing their repopulating ability and transplanted into a myeloablated host (2-6). As it is a very easy way to get the HSCs, CB has been the most frequent sample source of stem cells for the investigation of basic science and clinical research of stem cells.

The principal limitations of allogeneic BMT are the lack of suitable HLA-matched donors and the complications due to graft-versus-host disease (GVHD) that are more severe with increasing HLA disparities (7). Recent advances in the technique of HLA typing using DNA method reduced the probability of finding a fully matched donor although it improved the severity and incidence of GVHD (8). CB cells have many theoretical advantages as grafts for stem cell transplantation because of the immaturity of newborn cells. Compared to adults, UCB HSCs produce larger *in vitro* hematopoietic colonies, have different growth factor requirements, are able to expand in long-term culture *in vitro*, engraft severe combined immunodeficiency disease-human mice in the absence of additional human growth factors and have longer telomeres. The properties of UCB cells should theoretically compensate for the relatively low number of cells obtained in a single UCB unit and, through rapid expansion, reconstitute myeloablated patients.

Another advantage of UCB is the low incidence and severity of acute and chronic GVHD and it is related to the immaturity of the immune system at birth. This property decrease the alloreactive potential

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of the lymphocytes whithin a cord blood graft and as a consequence reduce the incidence and severity of GVHD after transplantation. Cord blood lymphocytes are said to be naive and immature and are enriched in double negative $CD3^+$ cells and produce fewer cytokines. CB cells express messenger RNA transcripts for interferon gamma, interleukin (IL)-4, and IL-10, but very little IL-2, have a fully constituted polyclonal T cell repertoire and can be protected from apoptosis because of low levels of CD95 (9,10). Most of these functions are inducible through *in vitro* or *in vivo* activation; as a consequence, early natural killer and T cell cytotoxicity is impaired, but secondary activation can occur. One can speculate that despite the reduction of GVHD, a graft-versus-leukemia effect can still be observed with UCB cells. Because acute GVHD is an early event after allogeneic BMT that is in part triggered by cytokine release, it is reasonable to postulate that UCB grafts might induce less frequent and less severe acute and chronic GVHD than adult HSC transplants that contain a greater number of activated T cells. These properties should lead to less stringent criteria for HLA donor recipient selection.

## Clinical Status of CBT

CBT offers the potential to increase the availability of stem cell to treat a variety of diseases and has shown several advantages over all BMT. It is immediately available and it has less HLA restriction for donors and it has lower risk of viral contamination of the graft and it has potentially reduced risk of GVHD (11). Currently there have been more than 2,000 CBT performed worldwide and more than 100 cases in Korea from related and unrelated donors to treat patients with malignant and non-malignant diseases (Table I-IV).

Clinical results of CBT are now available from many institutions worldwide and the two main registries are the International Cord Blood Transplant Registry and the Eurocord Registry. Two primary registries are based on the cord blood inventory of New York Blood Bank and Eurocord (Netcord).

*CBT from a related donor.* After the first case of CBT in Paris, more recent case of related CBT is a successful case from University of Minnesota in a 6-year-old girl with Fanconi's anemia. She received a CBT from sibling who was selected from *in vitro* fertilization after preimplantation genetic diagnosis. There are hot debates on the ethical problem of this case. Some people say that making a baby for the treatment of another baby is not ethical but others say that if there is no way to treat the child, it is a new way of treatment.

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

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

**Table II.** CBT in Korea

| | | | |
|---|---|---|---|
| ALL | 6 | HLA-matched | 4 |
| AML | 14 | HLA-mismatched | 28 |
| CML | 4 | 1 Ag | 8 |
| MDS | 1 | 2 Ag | 15 |
| SAA | 5 | 3 Ag | 5 |
| Genetic disease | 2 | | |

1996. 7～2002. 2: 39 patients

**Table III.** Clinical Data of CBT in Korea

| | GroupI (N=15) | Group II (N=17) |
|---|---|---|
| Age (mo) | 84 (8～144) | 20 (5～56) |
| BW (kg) | 21 (8～52) | 18 (10～50) |
| Infused cell | | |
| TNC ($\times 10^7$/kg) | 3.7 (2.5～13.2) | 3.8 (0.2～13) |
| CD34+ ($\times 10^5$/kg) | 1.9 (0.4～3.3) | 2.8 (0.4～9.4) |
| Engraftment | | |
| Neutrophil ($>1\times 10^3$/uL) | d37 (16～92) | d18 (10～37) |
| Platelet (>20,000/uL) | d78 (16～114) | d54 (15～91) |
| Survival (%) | | |
| OS | 60 | 70.6 |
| EFS | 53.3 | 58.2 |

Group I: Hospitals other than Catholic Univ, Group II: Catholic Univ Hospital

Table IV. Clinical Data of CBT in Korea

| (N=15) Median F-U duration 37.5 mo (2~68 mo) | | |
|---|---|---|
| Alive with disease free | | 7 |
| Alive with disease | | 4 |
| Death | | 4 |
| Persistent disease or relapse | 2 | |
| Graft failure | 1 | |
| Sepsis | 1 | |

*CBT from an unrelated donor*. CBT from an unrelated donor needs a CB banks which stores more than thousands of CB collected and donated for clinical use during delivery of the newborn. The pioneer of this CB bank is Dr. Rubinstein from New York Blood Center. The placental blood program at the New York Blood Center composed of 98 transplantation centers worldwide and provides the largest dataset of CBT from unrelated donors. As of 1998, cumulative rate of engraftment based on actuarial analysis for 562 patients were 81% for neutrophil recovery (median of 28 days) and 85% for platelet engraftment (median 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. Leukocyte 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. But the factor of age was significantly associated with event-free survival. This suggests that larger doses of leukocytes in CB may speed up engraftment but may not improve event-free survival, particularly for the older patients (12). As of January 2001, 831 children and adults had received CB from unrelated donors through the Eurocord Registry which was composed of 39 transplantation centers from 15 countries (13-15). Separate overall analyses were done for 291 children and 108 adults with malignant disease. The report of 291 children who received unrelated CB showed a 2-year event-free survival rate of 36% in patients with malignant diseases 21% in patients with aplastic anemia and 51% in patients with inborn errors; neutrophil engraftment by day 60 wss 82%; incidence of acute GVHD II IV in 39% of patients. Factors favorably associated with survival were ABO match and cytomegalovirus-negative status before CBT. 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. When only the 108 adults with malignancies were analyzed, overall survival rate at 1 year was 27%. Factors favorably associated with survival were cell dose and having a good risk status at the time of transplantation. The 1-year survival rate of poor-risk patients versus good-risk patient was 7% versus 36%. Median time to neutrophil recovery was 35 days. Median time to platelet recovery was 176 days. Acute GVHD more than grade II observed in 38% of patients was not associated with HLA disparities.

These studies indicate that CBT 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.

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

The threshold 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 less than $1.5\times10^7$/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.

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.

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.

## Immunological Properties of CB

The recent publication confirms earlier preclinial and clinical studis that suggested a loewr incidence of GVHD in CB stem and progenitor cell transplantation (16-18). It raises several issues about the immunologic properties of CB. Followings are summary of recent studies that show differences between CB immune cells and immune recovery after CBT. 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 (18-21). For example, CB T cells manifest less cytotoxic activity than adult T cells after primary, secondary, and tetiary allogeneic cell stimulation (22,23). 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 (24). The mechanisms of this tolerance of CB T cells to secondary allogeneic cell stimulation reflect the intracellular status of the CB T cell in that the inactive guanosine diphosphate (GDP)-bound form of Ras is not activated to the active guanosine triphosphate (GTP) form (25). More recent studies note that human CB has few or no cells with a $CD8^+$ NKT cell development (26). 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.

## Immune Reconstitution after CBT

Immune reconstitution after CBT is considered to be two steps. In the early post-transplant period, there is an expansion of mature donor-derived lymphocytes transferred with the graft. Thereafter, naive lymphocytes derived from the differentiation of donor HSCs colonize the lymphoid organs and sustain the late immune response of recipients. The first step of the immunologic recovery in CBT recipients could theoretically be expected to be less efficient compared to patients given BMT, due to the lower number of lymphocytes infused, which are also immature.

In a Eurocord study, risk factors influencing lymphocyte subset reconstitution related to disease, patient, donor and transplant were studied in 63 children (<16 years), given either related (n=14) or unrelated (n=49) UCBT for malignant (n=33) or non-malignant (n=30) diseases. Only children with sustained myeloid engraftment were analyzed. Absolute numbers of T ($CD3^+$, $CD4^+$, $CD8^+$), B and NK cells were reported 2～3, 6, 9, 12 and 12～24 months after UCBT. The median patient age was 4.0 years (0～15 years) and the median follow-up was 23 months (0～61 months.) Twenty-six patients received HLA-mismatched UCBT. The median number of nucleated cells collected/recipient weight was $6.1\times 10^7$/kg. In this selected population, the estimated 2-year survival was 85%. Lymphocyte reconstitution, defined as the median time to reach the normal value of age-matched healthy children, was 3, 6 and 8 months for NK, B and $CD8^+$ cells, while it was 11.7 months for both $CD3^+$ and $CD4^+$ lymphocytes. In multivariate analysis, factors favoring T cell recovery were: related donor ($P$=0.005) and recipient cytomegalovirus (CMV)-postive serology ($P$=0.04). The presence of acute GVHD delayed T cell recovery ($P$=0.04). To summarize, in children with sustained myeloid engraftment, the concern that lympnocyte recovery after CBT could be delayed does not appear to be substantiated by above results, and recovery of the absolute number of T cells in CBT recipients seems to substantially mimic that described in children given BMT.

Analysis of the CD45 molecule isofoms showed that usually CBT recipients have a greater percentage of $CD8^+/CD45RA^+$ compared to patients given BMT, whereas this difference in the distribution of CD45RA and RO antigens in less pronounce for the $CD4^+$ subpopulation. One study has suggested that, in patients given unrelated CBT, the recovery of $CD3^+/CD8^+$ lymphocytes is slower than that of BMT recipients. The proliferative response to polyclonal activators is comparable to the observed in BMT recipients. These findings suggest that, given the much lower number of lymphocytes transferred with CBT, when compared with BMT, the recovery of T lymphocyte number and function towards normal has to be considered to be rapid.

Functional cytotoxic specific activities (i.e. NK and LAK cytoxicity) of CBT recipients are comparable to those observed after BMT. A peculiar characteristic of the immune recovery in CBT recipients is represented by the expansion of B lymphocytes. In fact, in contrast to that observed in BMT recipients, an impressive increase in the percentage and absolute number of B lymphocytes, apparently not related to viral infections, has been documented in children receiving CBT. Possible hypotheses accounting for this phenomenon could involve the physiologic characteristics of B cell ontogeny in the first year of life and/or the different distribution of mature memory lymphocytes in bone marrow and cord blood.

Several crucial questions on the ability of CBT recipients to mount a T cell-mediated immune response towards widespread pathogens, the contribution of either donor or recipient origin immune cells to the antigen-specific immune response, the recovery and development of antigen presenting cells (APC) and the reconstruction of the T cell repertoire after CBT remain to be properly addressed.

## CB Banking

With the increased recognition that CB is a viable

source of HSCs 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 such as, rapid availability of CB, no donor risk or attrition, low risk of transmitting infectious diseases, potentially reducted risk of acute GVHD, and possible increased ability to expand the pool of donors to include ethnic and racial minorities.

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 May 2003, Bone Marrow Donors Worldwide (BMDW) estimated the current worldwide inventory of CB as 65,535 units, it is estimated that this number probably exceeds 80,000 units. This number is the sum of the 65,535 units listed by BMDW; the 14,000 units stored in 20 banks located in Japan, China, Korea, Thailand and Singapore 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 organization devoted to establishing quality, require addressing several issues, some of which have been successfully addressed and others that remain debated.

In Korea, some cord blood banks started to collect the CB of sibling of leukemia patients from 1998 but the actual business of CB banking started after the year of 2000 with the start of private CB banking of the famous movie star and sportsman. Several venture companies started this business, however it was not activated as the medical insurance does not cover the medical cost of CBT. Recently with the change of government policy to cover CBT by medical insurance, the necessity of public banking became aware by the medical doctors and the patients. But there are no regulations for the CB banking in processing, storing, distribution and there is no standards or quality assurance program for the stored CB.

## Conclusion

CBT 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 CBT 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 standadize banking to facilitate regulation, collection, processing, and distribution as a way of providing the highest-quality CB for patients use.

## References


1. Gluckman E, Broxmeyer HE, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Soie G, Lehn P, Cooper S, English D, Kurtzberg J, Bard J, Boyse EA: Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. New Engl J Med 321;1174-1178, 1989
2. Rubinstein P, Dobrila L, Rosenfield RE: Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 92;10119-10122, 1995
3. Rubinstein P, Rosenfield RD, Adamson JW, Stevens CE: Stored placental blood for unrelated bone marrow reconstitution. Blood 81;1679-1690, 1993
4. Gluckman E, Rocha V, Boyer Chammard A, Locatelli F, Arcese W, Pasquini R: Outcome of cord blood transplantation from related and unrelated donors. New Engl J Med 337;373-381, 1997
5. Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, Ciocci G, Carrier C, Stevens CE, Rubinstein P: Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. New Engl J Med 335;157-166, 1996
6. Wagner JE, Rosenthal J, Sweetman R, Shu XO, Davis SH, Ramsay NKC, McGlave PB, Sender L, Cairo MS: Successful transplantation of HLA matched and HLA mismatched umbilical cord blood from unrelated donor: analysis of engraftment and acute graft versus host disease. Blood 88; 795-802, 1996
7. Szydlo R, Goldman JM, Klein JP: Results of allogeneic bone marrow transplants for leukemia using donors other than HLA identical siblings. J Clin Oncol 15;1767-1777, 1997
8. Petersdorf EW, Gooley TA, Anasetti C: Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and the recipient. Blood 92;3515-3520, 1998
9. Henslee-Donney PJ, Gluckman E: Allogeneic transplantation from donors other than HLA identical siblings. Hematol/ Oncol Clin N Am 13;1017-1039, 1999
10. Madrigal JA, Cohen SBA, Gluckman E, Charron DJ: Does cord blood transplantation result in lower graft versus host disease? It takes more than two to Tango. Human Immunol 56;1-5, 1997
11. Rocha V, Wagner JE Jr, Sobocinski KA: Graft-versus-host disease in children who have received a cord blood or bone marrow transplant from an HLA-identical sibling. N Engl J Med 342;1846-1854, 2000
12. Rubinstein P, Carrier C, Scaradovou A: Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med 339;1565-1577, 1998
13. Gluckamn E, Rocha V, Boyer-Chammard A for the Eurocord Transplant Group and the European blood and Marrow Transplantation Group: Outcome of cord blood transplantation from related and unrelated donors. N Engl J Med 337;373-381, 1997
14. Gluckman E, Rocha V, Chastang C: Cord blood stem cell transplantation. Bail Clin Haemat 12;279-292, 1999
15. Gluckman E, Rochal V, Chastang C: European results of unrelated cord blood transplants. Bone Marrow Transplant 21(suppl 3);S87-S91, 1998
16. Broxmeyer HE, Douglas GW, Hangoc G: Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 86; 3828-3832, 1989
17. Broxmeyer HF, Kurtzberg J, Gluckman E: Umbilical cord

blood hematopoietic stem and repopulating cells in human clinical transplantation. Blood Cells 17;313-329, 1991
18. Broxmeyer HE, Smith FO: Cord blood stem cell transplantation. In: Forman SI, Blume KG, Thomas ED, eds. Stem Cell Transplantation. Cambridge, Mass: Blackwell Scientific Publication 431-443, 1999
19. Gaddy J, Broxmeyer HE: Cord blood natural killer cells: implication for cord blood transplantation and insights into natural killer cell differentiation. In: Broxmeyer HE, ed. Cellular Characteristics of Cord Blood and Cord Blood Transplantation. Bethesda, Md: American Association of Blood Banks 83-112, 1997
20. Gaddy J, Porcu P, Broxmeyer HE: Clinical and basic science studies of human umbilical cord blood transplantation. In: Barrett J, Jiang YZ, cds. Allogeneic Immunotherapy for Malignant Diseases. New York: Marcel Dekker 267-284, 2000
21. Smith FO, Thompson BG, Broxmeyer HE: Umbilical cord blood transplantation: current opinions in organ transplantation. Bone Marrow Transplant 5;358-365, 2000
22. Risdon G, Gaddy J, Stehman FB, Broxmeyer HE: Proliferative and cytotoxic responses of human cord blood T-lymphocytes following allogeneic stimulation. Cell Immunol 154;14-24, 1994
23. Risdon G, Gaddy J, Broxmeyer HE: Allogeneic responses of human umbilical cord blood. Blood Cells 20;566-572, 1994
24. Rison G, Gaddy J, Horic M, Broxmeyer HE: Alloantigen priming induces a state of unresponsiveness in human cord blood T cells. Proc Natl Acad Sci USA 92;2413-2417, 1995
25. Porcu P, Gaddy J, Broxmeyer HE: Alloantigen-induced unresponsiveness in cord blood T-lymphcytes is associated with defective activation of Ras. Proc Natl Acad Sci USA 95;4538-4543, 1998
26. Kim YJ, Broxmeyer HE: $CD8^+$ NKT cells, low in cord blood, preferentially require 4-1BB rather than CD28 for costimulation [abstract]. Blood. 96(suppl 1, pt 1);240a. Abstract 1031