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Umbilical cord blood: A promising source of stem cells

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Stem cells are cells that have the ability to self-replicate and give rise to specialized cells. Stem cells can be found at different stages of fetal development and are present in a wide range of adult tissues. Many of the terms used to distinguish stem cells are based on their origins and the cell types of their progeny. The hematopoietic stem cells are the most studied type of adult stem cells. Since mature blood cells are constantly lost and destroyed. Billions of new blood cells are produced each day to make up the loss. This process of blood cell generation called hematopoiesis occurs largely in the bone marrow. Thus the bone marrow is an established source of hematopoietic stem cells. However, in the past few years the human umbilical cord blood has emerged as a potential source of blood stem cells. The umbilical cord is a flexible cordlike structure connecting a fetus at the abdomen with the placenta and containing two umbilical arteries and one vein that transport nourishment to the fetus and remove its wastes. It has been observed that both term and preterm umbilical cord blood (UCB) contains a significantly large numbers of early and committed progenitor cells (3). These are called the umbilical cord stem cells.

Ontologically, hematopoiesis during embryonic and fetal development is represented as a migratory phenomenon. It is first extra-embryonic, in the yolk sac, and thereafter moves to intra-embryonic sites: first to the liver and spleen, and finally to the bone marrow (BM). Fetal blood immediately prior to delivery has been shown to contain hematopoietic progenitor cells at similar or higher levels than those in BM. Therefore UCB, which is normally discarded, has come to be valued as a source of stem/progenitor cells. When compared with adult peripheral circulation, the number of committed progenitor cells is higher in the umbilical cord blood (4). The number of colony-forming unit–granulocyte (CFU-GM) is greatly increased in umbilical cord blood obtained from term neonates compared with peripheral blood obtained from adults. The CFU-GM proliferative rate, as assayed by thymidine suicide studies, is also significantly higher in term umbilical cord blood (5). The number of circulating colony-forming unit granulocyte, erythroid, monocyte, megakaryocyte (CFU-GEMM) also appears to be significantly increased in term umbilical cord blood and the CFU-GEMM proliferative rate is also significantly compared with that of adult peripheral blood. Lastly, committed megakaryocytic progenitor cells as identified by circulating

colony-forming unit megakaryocyte (CFU-Meg) are also enriched in term umbilical cord blood compared with adult peripheral blood but to a much less severe degree than CFU-GM and CFU-GEMM (17).

Proliferation and Expansion of UCB Hematopoietic Stem/Progenitor Cells

The proliferation potential of Hematopoietic Stem/Progenitor Cells (HSPC) (defined as the capacity to divide and generate new daughter cells) as well as their expansion potential (defined as the capacity to produce more progenitor cells) appear to be biologic features that depend upon intrinsic factors. These are related to whether the cell is already committed or not to a particular lineage of differentiation and, if so, the specific hematopoietic lineage to which it belongs and its stage of maturation. However, the ability of a cell to exhibit such potentials depends on extrinsic factors that include the different cell types and cytokines that form part of the microenvironment in which the cell develops (10). In vitro proliferation and expansion of HSPC also depend on variables such as type of culture medium, medium change schedule, temperature, presence or absence of serum, number of cells plated per culture, etc. (14).

Several groups have addressed the in vitro expansion and proliferation of UCB progenitors using either total CD34+ cells or CD34+ cell subsets. In general, it is clear that primitive subpopulations of CD34+ cells possess greater expansion potential than their more mature counterparts. This has been observed in liquid cultures established in the presence of stromal cells or in the absence of such cells but in the presence of recombinant cytokines. Interestingly, it has been found that progenitor cells with high expansion capacity may show a lower proliferation potential than cells with a limited expansion capacity. Indeed, among the three CD34+ cell subpopulations identified on the basis of CD45RA and CD71 expression, CD34+ CD45RAlo CD71lo cells show the highest expansion potential, whereas CD34+ CD45RAlo CD71+ cells are the ones with the highest proliferation capacity (11).

The ability of HPC to express an intrinsic expansion and proliferation potential in vitro will depend on the cytokines present in culture. In this regard, several cytokines (including SCF, interleukin 1 (IL-1), IL-3, IL-6, GM-CSF, G-CSF, M-CSF, erythropoietin (Epo), thrombopoietin (Tpo), and FL have been assessed, either alone or in combination, in cultures of UCB-derived CD34+ cells (6,12,18,22). In terms of HPC expansion, the best results have been obtained when cytokines are used in combinations that include early-acting factors, such as SCF, FL, and Tpo. Indeed, the greatest expansion of UCB-derived CD34+ cells reported to date (146,000-fold expansion in CD34+ cell numbers and 2 x 10^6-fold expansion in CFC numbers) has been achieved by using both FL and Tpo (18). Addition of late-acting factors, such as Epo, usually contribute to the production

of large numbers of mature cells, however, they do not seem to have an effect on HPC expansion (11).

In contrast to the cytokines mentioned above, hematopoietic inhibitors, such as transforming growth factor-â, tumor necrosis factor-á, and macrophage inflammatory protein-1á have been shown to significantly reduce both expansion and proliferation of different CD34+ cell subpopulations from UCB (13). Indeed, some investigators have used antitransforming growth factor-b monoclonal antibody, together with stimulatory cytokines, to achieve a significant expansion of primitive progenitor cells.

Expansion and proliferation of UCB HPC has also been assessed in the presence of plasma both from UCB and adult peripheral blood (PB). Interestingly, it was found that UCB plasma induced significantly higher expansion/proliferation of CD34+ cells than adult PB plasma, suggesting that the former contains a factor(s), absent in PB plasma, important for UCB HPC growth (20). It has also been reported that expansion and proliferation of UCB HPC is influenced by the cell density in culture. *Xiao et al*. compared the expansion and proliferation capacity of CD34+++ cells when plated at 1 or 5,000 cells per well, in the presence of recombinant cytokines. They found that proliferation was greater in cultures of single cells, whereas expansion was favored in cultures of 5,000 cells (23). They suggested that a feeder effect occurred when CD34+++ cells were plated at higher cell densities and that either cell-cell contact was necessary, probably due to stimulation from membrane-bound cytokines, or that cytokines in addition to those supplied were required for optimal progenitor cell expansion.

Applications

Hematopoietic cell transplantation (HCT) can be curative for selected malignant and nonmalignant diseases like Fanconi's anemia, aplastic anemia, leukemias, metabolic and other congenital disorders. However, utilization and success of HCT are limited by several obstacles, primarily related to the importance of donor-recipient genetic match for favorable outcomes. While in most settings, best results are offered by human leukocyte antigen (HLA) identical sibling transplantations, more than two thirds of patients awaiting HCT lack a suitable related donor. The availability of haematopoietic stem or progenitor cell donors is a global challenge. Even in the western world, despite over 9 million registered donors, over 50% of patients needing HLA matched donors are unable to get one. Also, the time taken from initiating search to transplantation is 6 months or more.

The recent use of either related umbilical cord blood or unrelated donor umbilical cord blood stem cells for allogeneic stem cell transplantation has been secondary to a number of factors, most important of which have been (1) the attempt to reduce transplant-related complications and (2) augmentation of the donor pool. Gluckman in 1988 first reported the successful use of HLA-matched sibling umbilical cord blood stem cells to reconstitute a child with severe Fanconi anemia. Since 1988, there have been an estimated 500 related and unrelated donor umbilical cord blood transplants. Current data indicate that HLA mismatch may be better tolerated in the UCBT setting than BMT. A study comparing outcomes of HLA-identical sibling UCBT versus HLA-identical sibling BMT in pediatric recipients observed significantly lower incidence of acute and chronic graft versus host disease (GVHD) in the UCBT group. This is perhaps the clearest demonstration of a difference in biologic properties between the 2 stem cell sources. Data suggests that the engraftment could occur despite mismatching at two loci, that the risk of severe GVHD was low, and that a higher cell dose is important. Four strategies have been developed to improve the outcomes: 1) Pooled or sequential cord blood transplantation 2) Cord blood expansion 3) Combined cord blood and haplo-identical bone marrow transplants 4) Non-myeloablative or reduced intensity conditioning regimens with 1, 2 or 3.

While autologous transplantation of umbilical cord stem cells is still an investigational tool. Autologous umbilical cord blood hematopoietic stem cells potentially could be used to correct genetic deficiencies at birth of hematopoietic stem cells after successful gene transduction and autologous umbilical of cord blood stem cell transplantation. Using retroviral vectors containing a neomycin-resistant gene or cDNA encoding adenosine deaminase (ADA), UCB cells were studied for transduction efficiency. Results showed that UCB cells were more efficiently transduced than BM cells. Ongoing and future studies must still evaluate UCB cells as candidates for cellular vehicles of gene therapy.

Advantages of UCB

UCB cells have been reported to have several advantages over BM cells in terms both of proliferative capacity and immunologic reactivity (Table 1). Since no assay is available that can identify the pluripotential of hematopoetic cells because of the immense heterogeneity of the cells within the stem and progenitor cell compartments, therefore, surrogate assays have included the expression of cell surface antigens (CD34, HLADR, CD38, CD71, CD45RA, Thy-1), long-term culture-initiating cell (LTC-IC), high proliferative potential colony-forming cell (HPP-CFC) and colony-forming cell (CFC) assays. Though the content of CD34+ cells indicate a similar frequency of CD34+ cells in BM and UCB, accounting for approximately 1% of the nucleated cells in the

unfractionated sample. A primitive subpopulation of CD34+ cells not expressing CD71 and CD45RA (CD34+CD71– CD45RA–) has been reported to represent 25% of UCB CD34+ cells as compared with 5-20% of CD34+ BM cells and contain up to 42% of multipotent progenitors. Inspite of the fact that UCB cannot be established in primary long-term culture because it lacks sufficient stromal precursor cells to provide the microenvironment necessary for self-renewal and differentiation of hematopoietic cells, when a preformed stroma layer is provided the amplitude and length of progenitor cell production from UCB is superior to that of normal BM.

UCB, which is normally discarded, is easily collected at the time of delivery without any danger or inconvenience to the donor or to the mother (7). Collection can be accomplished by venipunction of the umbilical vein: i) with the placenta still in utero, or ii) after the delivery of the placenta itself. A major advantage of cord blood is the speed of the search process as there is no living donor to contact and retest (2).

Following UCB transplants the GVHD is milder and the incidences are also lower (9). This might be because hematopoiesis and host defense in the neonate is developmentally immature compared with the adult. Significant dysregulation of a number of hematopoietic cytokines and lymphokines from umbilical cellular sources compared with adult peripheral blood have been demonstrated. The proliferative response after allogeneic stimulation of E-rosetted T cells is similar in umbilical cord blood compared with adult peripheral blood. However, in contrast, allogeneic cytolytic activity in mixed peripheral blood MNC. Dysregulation of hematopoietic leukocyte cultures of umbilical cord blood T cells is significantly decreased compared with that in adult peripheral blood.

Table 1. **UCB stem cells: potential advantages.**

Biological
High proliferative capacity
Low risk of viral contamination
Defective cytotoxic response to alloantigens

Clinical
Availability
Donor safety
Ethnic balance
Reduction in unrelated donor search time
Absence of donor attrition

Genetic Manipulation of UCB Cells

Genetic manipulation of human cells (including Hematopoietic Stem/Progenitor Cells) has been of great biomedical interest because of its potential relevance in the treatment of specific disorders (15). Several investigators have reported on the successful introduction of particular genes into primitive hematopoietic cells from BM (21) and similar approaches are being used with UCB cells. *Moritz et al*. compared the efficiency of gene transfer into both UCB and BM MNC. Two genes were analyzed, TK-neo (which confers resistance to the drug G418, a neomycin analog) and adenosine deaminase (ADA), involved in the development of SCID. Both genes were successfully transferred into primitive HPC (15). Interestingly, these authors observed that the proportion of cells resistant to G418 was significantly higher in UCB cultures than in similar cells from BM. These results indicate that UCB HPC are better targets for gene transfer than HPC from BM, and suggest that UCB cells may have distinct advantages in some protocols of human gene therapy. Genetically manipulated CD34+ UCB cells have been recently used in the treatment of patients with SCID (8). Three women who were known to be heterozygous for ADA deficiency were identified to be carrying ADA-deficient fetuses. After term delivery of each neonate, UCB was collected, and CD34+ cells were isolated by standard methods. The CD34+ cells were transduced with the retroviral vector LASN, which carried the TK-neo gene and a normal human ADA cDNA. On the fourth day after birth, the transduced CD34+ cells were reintroduced to their respective donors by i.v. infusion. The efficiency of retroviral-mediated transduction of clonogenic myeloid progenitors contained within the UCB cells before transplantation was assessed by colony assays. From the three patients, 21.5%, 12.5%, and 19.4% of the myeloid progenitor cells were resistant to G418. After 18 months post transplant, all three patients showed the presence of BM granulocytes and MNC containing the LASN vector at approximately 1/10,000 cells. A similar frequency was observed in leukocytes present in the PB. Myeloid progenitors were cultured to assess the expression of the retroviral vector, and it was found that 4% to 6% of myeloid CFC were G418-resistant. According to this report, there have been no adverse effects from the administration of gene-modified UCB cells after 18 months post transplant. The continued administration of ADA enzyme replacement therapy has allowed the patients to develop normal immune function and to remain free of infections (8). Obviously, in this study, SCID patients were not cured by the introduction of genetically manipulated UCB; however, these results are very encouraging, since they demonstrate the feasibility of using genetically modified UCB cells in clinical settings.

Cord Blood banking

Over the past few years, umbilical cord blood has moved from the status of biologic waste to a potentially important source of hematopoietic stem cells. As a result the concept of cord blood banking has come up. Cord blood banking involves recruitment, consent, testing of maternal donors, collection, processing, cryopreservation, testing, and releasing cord blood unit to transplant centre. Cord blood banking is of two types i.e. public and private. A woman can donate cord blood for unrelated recipient to public banks (unrelated allogeneic transplantation). Private (commercial) banks, on the other hand, offer expectant parents the option to store cord blood for possible future use by that same child (autologous transplantation).The first cord blood bank was established in early 1990s in New York (19). Subsequently, several cord blood banks have come up in several countries. Most of the cord blood units are typed for HLA A, B and DR; 75% are molecular typed for class II and 50% are molecular typed for class I (1). While this approach has been highly successful, with permission to collect UCB in 91% of cases, the anonymous nature of storage makes it impossible to trace the donor at the time of transplant in order to acquire further information about the health of the donor during infancy.

There is already a debate whether commercial cord blood banking is scientifically and ethically justified. Questions are being raised whether the commercial banking exploits the emotional vulnerabilities of parents for financial gain. Parents often believe or are made to believe that the child's own stem cells are the best. It is also possible that the private industry might overlook important safety issues. The investigational nature of autologous umbilical cord stem cell transplant may not be openly discussed. That the genetic disorders cannot be cured by autologous transplants may not be emphasized. The duration for which the cells can be stored is questionable. Benefits are often overstated. Untruthful advertising is common. Discloser of uncertainties is rare. Cost involved is large and full discloser of financial aspects is often not made.

ETHICAL ISSUES

The collection and use of umbilical cord blood has opened the way for several unresolved ethical issues. If the UCB is considered to be like any other organ donation then the consent of the donor is necessary and in this case the donor is a minor leaving the decision to parents. In this case when should the consent be obtained and what would be right of the donor (the child) after he becomes a major?. Several religion and communities may not allow the collection of placental tissues. After collection the results of the preliminary tests may reveal infectious or congenital

genetic diseases like HIV, thallesmia etc .Such results should be informed to the donor or not? Ethical issues are not limited to the area of the consent and disease screening; other issues include (1) the potential of deliberate conception and embryo cloning for producing an HLA-identical tissue donor and (2) commercialization. These issues need to be considered by medical ethicists and various members of the medical community so that a consensus opinion might be made available to help guide clinicians and storage services in their practices.

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