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UMBILICAL CORD BLOOD AS A SOURCE OF HEMATOPOIETIC STEM CELLS: FROM RESEARCH TO CLINICAL APPLICATION

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ABSTRACT

The clonogenic capacity of human umbilical cord blood (UCB) has been evaluated in several studies which found high numbers of primitive hematopoietic progenitor cells. Recently, UCB progenitor cells were shown to possess significant advantages over bone marrow (BM) in terms of proliferative capacity and immunologic reactivity. Therefore UCB has come to be considered an attractive source of hematopoietic stem cells for both research and clinical applications. UCB has been used in the treatment of diseases potentially curable by bone marrow transplantation (BMT). Seventy-one transplants have been performed world-wide using UCB cells, and the results have been reported to the International Cord Blood Transplant Registry (ICBTR). Since UCB cells appear to be less alloreactive than BM cells, studies are under way to determine the feasibility of UCB banking for use in unrelated transplants. Because of the limited volume of UCB that can be obtained in a single collection, studies have been carried out to determine the most successful procedures for collection and fractionation of UCB and to quantify precisely the progenitor/stem cell content.

The different techniques for quantifying progenitor/stem cells as well as the results of related and unrelated UCB transplants will be reviewed. Further clinical applications of UCB involving gene therapy and stem cell expansion will be discussed.

Key words: umbilical cord blood, hematopoietic stem cells, transplantation

The structural and functional integrity of the hematopoietic system is maintained by a relatively small population of stem cells that undergo self-renewal or differentiation into lineage-restricted progenitors.[1-8] Bone marrow (BM) is the primary site of hematopoietic stem cells in adults[2] but these cells have been documented in peripheral blood (PB),[9-13] and more recently they have been extensively studied in umbilical cord blood (UCB).[14-16]

Ontologically, hematopoiesis during embryonic and fetal development is represented as a migratory phenomenon. It is first extraembryonic, in the yolk sac, and thereafter moves to intraembryonic sites: first to the liver and spleen, and finally to the bone marrow.[17-19] Fetal blood immediately prior to delivery has been shown to contain hematopoietic progenitor cells at similar or higher levels than those in BM.[14-16,20-23] Therefore UCB, which is normally discarded, has come to be valued as a source of stem/progenitor cells. It can be easily collected without any danger or inconvenience to the donor[24] and can be used as an alternative to BM for clinical transplantation.[25-28]

Preliminary data suggest that UCB cells are less alloreactive than BM cells,[29] and studies are under way to determine the feasibility of UCB banking for use in unrelated transplants.[24] UCB is an attractive source of transplantable cells that can be used in the treatment of diseases that are potentially curable by bone marrow transplantation (BMT) (e.g. Fanconi's anemia, aplastic anemia, leukemias, metabolic and other congenital

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disorders).[28]

More recently, UCB-derived progenitor cells have been reported to possess significant advantages in terms of proliferative capacity and immunologic reactivity,[30-33] making them ideal candidates for experimental programs involving gene transfer and *ex vivo* stem cell expansion.

Advantages of UCB

UCB cells have been reported to have several advantages over BM cells in terms both of proliferative capacity and immunologic reactivity. Therefore UCB banks could have critical advantages over volunteer BM donor registries (Table 1).

Progenitor cell content

Many efforts have been made to quantify the progenitor cell content in BM, mobilized PB and UCB samples. Unfortunately no available assays are capable of identifying pluripotential hematopoietic stem 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, HLA-DR, 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. However, these assays are poorly standardized between laboratories, making direct comparison very difficult.

Data on 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.[34] In an attempt to better characterize a more primitive subpopulation of cells, the CD34 antigen has been coupled with CD38, CD45RA, CD71, HLA-DR and Thy-1 expression. A primitive subpopulation of $CD34^+$ cells not expressing CD71 and CD45RA ($CD34^+CD71^-CD45RA^-$) has been reported to represent 25% of UCB $CD34^+$ cells (compared with 5-20% of $CD34^+$ BM cells) and contain up to 42% of multipotent progenitors.[30, 31] Further selection of these purified cells using Thy-1 expression resulted in a significant enrichment in HPP-CFC with respect to the Thy-1 negative counterpart.[35]

In contrast to adult BM, where the primitive progenitors reside in the HLA-DR- compartment, UCB primitive progenitors are equally distributed in both the $HLA\text{-}DR^+$ and $HLA\text{-}DR^-$ fractions.[32] Moreover, self-renewal as a characteristic of more primitive progenitor cells has been assessed by replating CFU-GEMM,[14,15,20] and the results showed that in secondary cultures initiated by single $CD34^+$ cells in the presence of SCF, UCB contains about eight times more HPP-CFC than BM.[33] The LTC-IC assay has been used to detect an earlier cell than the HPP-CFC.

It has been reported 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 hemopoietic cells, while, on the contrary, if a preformed stroma layer is provided the amplitude and length of progenitor cell production from UCB is superior to that of normal BM.[21] When a limiting dilution assay is employed, the LTC-IC frequency has been reported to be identical in BM, mobilized PB and UCB.[23]

In summary, committed hematopoietic progenitors (CFU-GM, BFU-E) are present in UCB in numbers at the lower end of the normal range for BM; earlier progenitors (CFU-GEMM, HPP-CFC) are more frequent than in BM, while LTC-IC are reported to have the same frequency as in BM but with a greater capacity to form CFU-GM.[23]

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

Collection

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.[24,36] 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. The first method[14,37,38] takes advantage of uterine contractions, but must be done rapidly and may interrupt the obstetrician's routine activities and, moreover, is subject to contamination by maternal blood or microorganisms. The latter method[24] might reduce contamination, but on the other hand is burdened by a higher risk of collapse of umbilical vein and its tributaries. As anticoagulant, heparin and ACD (acid, citrate, dextrose) have been utilized with good results; CPD (citrate, phosphate, dextrose), however, was recently proven to be less affected by variations in collected blood volume and therefore offers an advantage for UCB collection.[24]

Separation

Physical separation of mononuclear cells and depletion of red blood cells (RBC) have been reported to result in significant losses of progenitor cells,[14,39] and therefore until recently the recommendation of the *New York Cord Blood Bank* had been to freeze the whole UCB collection without fractionation. Of late,[36,38] a specific separation procedure for UCB using gelatine sedimentation has been tested with good results. We reported significant results in terms of RBC depletion and recovery of progenitor cells utilizing sedimentation over poligeline.[40] Working with poligeline is easy and safe, since a poligeline solution is commercially available (Emagel, Behringwerke, Marburg, Germany), unlike gelatine, which comes in powder form and needs to be house-prepared and autoclaved for sterilization before use. Sedimentation over either gelatine or poligeline allows an efficient depletion of RBC without affecting progenitor cell recovery. These separation procedures would permit a sensible reduction in the volume of samples that need to be cryopreserved, thus reducing storage space and lowering the costs of banking.

Cryopreservation

Dimethyl-sulphoxide is used most often as the cryoprotectant and the cooling procedure is frequently carried out by a computerized controlled-rate freezer. Frozen UCB units are then kept in liquid or vapor phase nitrogen. Cryopreservation affects neither recovery nor the clonogenic capacity of hematopoietic progenitor cells in unseparated frozen UCB for up to 7 years of storage.[38] We tested the clonogenic capacity of mononuclear UCB cells before cryopreservation and after thawing, and achieved a recovery of 82% for CFU-GEMM, 94% for BFU-E, 82% for CFU-GM and 90% for more primitive progenitor cells as tested in long-term culture.[40]

Alloreactivity of UCB cells

The relatively low incidence and mildness of GVHD following UCB transplants have triggered studies aimed at exploring the in vitro immune reactivity of UCB cells.[29,41] Purified UCB T lymphocytes have shown a proliferative response to allogeneic antigens in mixed lymphocyte culture equal to that of adult peripheral blood lymphocytes (PBL), but a reduced capacity to stimulate allogeneic cells. This latter effect could have been related to either reduced expression of class II antigens or a reduced antigen-presenting capacity of monocytes.[42] Natural killer and lymphokine-activated killer activity have been reported to be equivalent to adult PBL,[29] but the impact in terms of graft-versus leukemia activity remains to be proven.[43] Studies are still needed which fully elucidate the differences in the immunoreactivity of UCB cells as compared to that of adult peripheral blood cells.

Cord blood banking

Several programs throughout the world are currently evaluating the feasibility of large-scale UCB banking for unrelated transplants, with fairly different approaches. The New York Blood Center (NYBC) has decided to store UCB samples after pregnant mothers have signed a consent form allowing disposal of the placenta, which becomes the property of the hospital.[24] 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. As of May 1995, 4600 UCB samples have been collected at the NYBC. In contrast, the European Cord Blood Bank[44] decided to bank UCB only after consent has been obtained from the mother that will include *back-tracking*. Exclusions from donation will be made on the basis of a family history of inherited diseases, complicated pregnancy or delivery, congenital abnormalities or absence of informed consent. A promising program on a national basis is represented by the Italian Cord Blood Bank Network,[36] which joins different groups (Milan, Bologna, Florence, Padua, Parma, Pavia, Rome and Turin) in an effort to standardize collection, HLA-typing, quality control and cryopreservation procedures. The goal is to store 5000 UCB samples over the next three years.

Clinical results

To date, seventy-one transplants have been performed world-wide using UCB either from siblings or unrelated donors and data concerning graft composition and hemopoietic recovery have been reported to the International Cord Blood Transplant Registry (ICBTR).[45]

Sibling UCB donors

Cryopreserved umbilical cord blood from sibling donors has been used to reconstitute hematopoiesis in fifty children with malignant and non-malignant diseases (Table 2). Median age of patients was 4.7 years and body weight 19 kg. Thirty-nine donor-recipient pairs were HLA-identical, four mismatched for one antigen, 1 for two antigens and six for three antigens. The median time to hematopoietic recovery was 22 days for ANC $\geq 0.5\times10^9$/L and 48 days for platelets $\geq 50\times10^9$/L (Table 3). The characteristics of the grafts are reported in detail in Table 4. No correlations were found between time to engraftment and nucleated cells/kg or CFU-GM/kg infused. Growth factors were utilized in about two-thirds of the patients (n=19) but showed no effect on the time to neutrophil recovery (p=0.12). The probability of grade II-IV acute graft-versus-host disease (GHVD) at 100 days post-transplant was 0.02±0.02 (95% CI, 0.00 to 0.08), with no patient presenting grade IV acute GVHD. Only one patient with an HLA-3 antigen disparate graft developed steroid resistant grade III acute GVHD. The probability of chronic GVHD at one year in evaluable patients was 0.06±0.04 (95% CI, 0.00 to 0.15), with no patient exhibiting extensive disease.

Table 2. Patients receiving UCB transplants from sibling donors reported to the ICBTR.

Disease	*n (50)*
Malignant disease	
Acute lymphoblastic leukemia	15
Acute myeloblastic leukemia	9
Juvenile chronic myelogenous leukemia	3
Chronic myelogenous leukemia	1
Neuroblastoma	2
Non-malignant disease	
Fanconi anemia	6
Severe aplastic anemia	5
β-thalassemia	2
Sickle cell anemia	1
Severe combined immune deficiency	1
X-linked lymphoproliferative syndrome	1
Wiskott-Aldrich syndrome	1
Mucopolysaccharidosis	3

Table 3. Characteristics of patients receiving UCB transplant.

Median age in years	4.7
Median weight in Kg	19
Degree of HLA-antigen match	
none	39
1-antigen mismatch	4
2-antigen mismatch	1
3-antigen mismatch	6
Recovery (days)	
ANC $\geq 0.5\times10^9$/L	22 (12-46)
PP $\geq 50\times10^9$/L	48 (15-100)

Unrelated UCB donors

Twenty-one patients with malignant and non-malignant diseases received a UCB trans-

Table 4. Characteristics of UCB collections used for transplant.

	Median	*Range*
Volume (mL)	100	41-282
Total cells ($\times 10^8$)	7.8	2-73
Cells/kg ($\times 10^7$)	5	1-33
Total CFU-GM ($\times 10^5$)	4.2	0.01-128
CFU-GM/kg ($\times 10^4$)	1.9	0.01-100

plant from an unrelated donor through cord blood banks (Table 5). These data represent a preliminary report of ICBTR results and therefore definitive considerations are still premature; however, promising future applications of unrelated transplantation can be hypothesized. Three patients received UCB from HLA-identical donors, thirteen from 1-antigen, four from 2-antigen and one from 3-antigen mismatched donors. Four patients died too soon to evaluate engraftment, two grafts failed. Fifteen patients are evaluable for GVHD: three had no GVHD, ten grade II and two patients grade III GHVD. Six patients died of infection, one from persistent leukemia, and fourteen are still living.

Future perspectives

UCB expansion

Major concerns about wider transplant application of UCB have been related to the low total number of progenitor cells that can be obtained in a single collection. Concerns are even higher when the recipient is an adult patient. Therefore, many attempts have been made to evaluate the possibility of expanding *ex vivo* stem and progenitor cells.[16,20,30-33,35,39,46] Since we lack a specific assay for testing human long-term repopulating cell, indications of early progenitor cell content are derived from expression of certain cell surface antigens (CD34, CD38, HLA-DR, CD71, CD45RA, Thy-1) and from the numbers of CFC, HPP-CFC and LTC-IC. *Ex vivo* expansion has been performed with different cytokine combinations and with the use of bioreactors;[47] other variables have included the presence of feeder layers and the effect of stroma non-contact.[16,21,30-32] Recently, telomere length was described as a biomarker of cellular age,[48] and it was proposed that the sequential loss of telomeric DNA from the ends of human chromosomes with each somatic cell division eventually reaches a critical point that triggers cellular senescence.[49] It has been reported that $CD34^+CD38lo$ stem cells purified from adult BM have shorter telomeres than cells from UCB or fetal liver, thereby signalling a difference in replicative capacity.[50] Results to date indicate that UCB has a greater ability to expand $CD34^+$ cells and CFU-GM content than BM; however, only limited expansion could have been detected in LTC-IC numbers.

Gene therapy

Retroviral-mediated gene transfer for correction of genetic disorders has been studied in murine and primate hematopoietic cells,[51] and is currently under clinical evaluation with adult BM and PB. Using retroviral vectors containing a neomycin-resistant gene or cDNA encoding adenosine deaminase (ADA), UCB cells were studied for transduction efficiency. Expression of the transduced gene was studied in CFU-GM and in LTC-IC, and results showed that UCB cells were more efficiently transduced than BM cells.[52,53] At this time, three children with ADA deficiency have received infusions of autologous UCB $CD34^+$ cells that were transduced with an ADA-containing retrovirus.[28]

Definitive results on the stability of gene integration, engraftment of the transduced cells and

Table 5. Patients receiving UCB transplants from unrelated donors reported to the ICBTR.

Disease	*n (21)*
Malignant disease	
Acute lymphoblastic leukemia	10
Acute myeloblastic leukemia	6
Chronic myelogenous leukemia	1
Non-malignant disease	
Fanconi anemia	2
Severe aplastic anemia	1
Globoid cell leukodystrophy	1

expression of the inserted genes are not yet available. Ongoing and future studies must still evaluate UCB cells as candidates for cellular vehicles of gene therapy.

Conclusions

UCB has proved to be an important source of hematopoietic progenitor/stem cells, and transplantation results support its use as an alternative to BM. Median time to hematopoietic recovery after UCB grafts is reported to be only slightly delayed with respect to allogeneic peripheral blood stem cells or BM, probably due to either the immaturity or the lower number of progenitors reinfused. UCB banks would have many practical advantages over volunteer BM donor programs, such as the *National Marrow Donor Program* in the US and the *International Bone Marrow Registry*. In fact, UCB cells frozen and stored in banks could be made available on demand, thus eliminating the delays that now complicate the search for a BM donor, while the large number of potential UCB donors would overcome ethnic imbalances. Moreover, UCB banks would not have to depend on the recruitment and continued collaboration of large numbers of potential volunteer donors, nor would they need to compensate for the attrition caused by retired volunteers. Therefore UCB banks could be a natural complement of BM donor registries in the future. As more collections are made, UCB harvesting will become a familiar aspect of the delivery room. Ongoing laboratory studies will determine optimal collection, separation and purification methods, while research is still necessary to broaden our knowledges about the immunological reactivity of subsets of UCB cells.

References

1. McCulloch EA. Stem cells in normal and leukemic hemopoiesis. Blood 1983; 62:1-13.
2. Ogawa M, Porter P, Nakahata T. Renewal and commitment to differentiation of hemopoietic stem cells (an interpretative review). Blood 1983; 61:823-9.
3. Carlo Stella C. Biological aspects and clinical applications of long-term bone marrow culture: a meeting report. Haematologica 1993; 78:236-8.
4. Torok-Storb B. Cellular interactions. Blood 1988; 72:373-85.
5. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. Science 1987; 236:1229-37.
6. Broxmeyer HE, Vadhan-Raj S. Preclinical and clinical studies with the hematopoietic colony stimulating factors and related interleukins. Immunol Res 1989; 8:185-201.
7. Moore MAS. Clinical implications of positive and negative hematopoietic stem cell regulators. Blood 1991; 78:1-19.
8. Carlo-Stella C, Rizzoli V. Stem cells and stem cell factor(s). Haematologica 1995; 80:1-4.
9. Kessinger A, Armitage JO, Landmark JD, Smith DM, Weisenburger DD. Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy. Blood 1988; 71:723-7.
10. To LB, Juttner CA. Peripheral blood stem cell autografting: a new therapeutic option for AML. Br J Haematol 1987; 66: 285-8.
11. Bell AJ, Figes A, Oscier DG, Hamblin TJ. Peripheral blood stem cell autografting. Lancet 1986; i:1027.
12. Juttner CA, Dyson PG, To LB, Ho JQK, Haylock DN, Roberts MM. Autologous blood stem cell transplantation in acute myeloid leukemia. Lancet 1985; i:419-20.
13. Siena S, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM. Circulation of $CD34^+$ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. Blood 1989; 74:1905-14.
14. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 1989; 86:3828-32.
15. Broxmeyer HE, Kurtzberg J, Gluckman E, et al. Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation. Blood Cells 1991; 17:313-29.
16. Broxmeyer HE, Hangoc G, Cooper S, et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation of adults. Proc Natl Acad Sci 1992; 89:4109-13.
17. Moore MAS, Metcalf D. Ontogeny of the hematopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryos. Br J Haematol 1970; 18:279-96.
18. Tavassoli M. Embryonic and fetal hemopoiesis: an overview. Blood Cells 1991; 1:269-81.
19. Migliaccio G, Migliaccio AR, Petti S. Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac to liver transition. J Clin Invest 1986; 78:51-60.
20. Carow CE, Hangoc G, Cooper SH, Williams DE, Broxmeyer HE. Mast cell growth factor (c-kit ligand) supports the growth of human multipotential progenitor cells with a high replating potential. Blood 1991; 78:2216-21.
21. Hows JM, Bradley BA, Marsh JCV, et al. Growth of human umbilical cord blood in long-term hemopoietic cultures. Lancet 1992; 340:73-6.
22. Nicol AJ, Hows JM, Bradley BA. Cord Blood transplantation: a practical option? Br J Haematol 1994; 87:1-5.
23. Pettengel R, Luft T, Henschler R, et al. Direct comparison by limiting dilution analysis of long-term culture-initiating cells in human bone marrow, umbilical cord blood, and blood stem cells. Blood 1994; 84:3653-9.
24. Rubinstein P, Rosenfield RE, Adamson JW, Stevens CE. Stored placental blood for unrelated bone marrow reconstitution. Blood 1993; 81:1679-90.
25. Gluckman E, Broxmeyer HE, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi anemia by means of umbilical cord blood from a HLA identical sibling. N Engl J Med 1989; 321:1174-8.
26. Wagner JE, Broxmeyer HE, Byrd RL, et al. Transplantation of

umbilical cord blood after myeloablative therapy: analysis of engraftment. Blood 1992; 79:1874-81.
27. Wagner JE. Umbilical cord blood stem cell transplantation: current status and future prospects. J Hematother 1993; 2: 225-8.
28. Apperley JF. Umbilical cord blood progenitor cell transplantation. Bone Marrow Transpl 1994; 14:187-96.
29. Harris DT, LoCascio J, Besencon FJ. Analysis of the alloreactive capacity of human umbilical cord blood: implications for graft-versus-host disease. Bone Marrow Transplant 1994; 14: 545-53.
30. Lansdorp PM, Dragowska W, Mayani H. Ontogeny related changes in proliferative potential of human hematopoietic cells. J Exp Med 1993; 178:787-91.
31. Mayani H, Dragowska W, Lansdorp PM. Characterization of functionally distinct subpopulations of CD34 positive cord blood cells in serum free long term cultures supplemented with hematopoietic cytokines. Blood 1993; 82:2664-72.
32. Traycoff CM, Abboud MR, Laver J, Brandt JE, Hoffman R, Law P, Ishizawa L, Srour EF. Evaluation of the in vitro behavior of phenotypically defined populations of umbilical cord blood hematopoietic progenitor cells. Exp Hematol 1994; 22: 215-22.
33. Lu L, Xiao M, Shen RN, Grisby S, Broxmeyer HE. Enrichment, characterization, and responsiveness of single primitive $CD34^{+++}$ human umbilical cord blood hematopoietic progenitors with high proliferative and replating potential. Blood 1993; 81:41-8.
34. Kinninburgh D, Russell NH. Comparative study of CD34 positive cells and subpopulations in human umbilical cord blood and bone marrow. Bone Marrow Transplant 1993; 12: 489-94.
35. Mayani H, Lansdorp PM. Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood. Blood 1994; 83:2410-7.
36. Bertolini F, Lazzari L, Lauri E, et al. A comparative study of different procedures for the collection and banking of umbilical cord blood. J Hematother 1995; in press.
37. Wagner JE, Broxmeyer HE, Cooper S. Umbilical cord and placental blood hematopoietic stem cells: collection, cryopreservation and storage. J Hematother 1992; 1:167-73.
38. Harris DT, Schumacher MJ, Rychlik S, et al. Collection, separation and cryopreservation of umbilical cord blood for use in transplantation. Bone Marrow Transpl 1994; 13:135-43.
39. Migliaccio G, Migliaccio AR, Druzin ML, Giardina PJV, Zsebo KM, Adamson JW. Long-term generation of colony-forming cells in liquid culture of CD34 positive cord blood cells in the presence of recombinant human stem cell factor. Blood 1992; 79:2620-7.
40. Almici C, Carlo-Stella C, Mangoni L, et al. Density separation of umbilical cord blood and recovery of hemopoietic progenitor cells: implications for cord blood banking. Stem Cells 1995; in press.
41. Harris D, Schumacher MJ, Locascio J, et al. Phenotypic and functional immaturity of human umbilical cord blood lymphocytes. Proc Natl Acad Sci USA 1992; 89: 10006-10.
42. Deacock SJ, Schwarer AP, Bridge J, Batchelor JR, Goldman JM, Lechler R. Evidence that umbilical cord blood contains a higher frequency of HLA class II-specific alloreactive T cells than adult peripheral blood. Transplantation 1992; 53:1128-34.
43. Slavin S, Ackerstein A, Naparstek E, Weiss L, Or R. The graft-versus-leukemia (GVL) phenomenon: is GVL separable from GVHD? Bone Marrow Transplant 1990; 6:155-61.
44. Gluckman E, Wagner JE, Hows J, Kernan N, Bradley B, Broxmeyer HE. Cord blood banking for hematopoietic stem cell transplantation: an international cord blood transplant registry. Bone Marrow Transplant 1993; 11:199-200.
45. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical cord blood transplantation in forty-four children with malignant and non-malignant disease. Lancet 1995; in press.
46. Almici C, Carlo-Stella C, Wagner JE, et al. Immunoselection of CD34 positive cells from umbilical cord blood and cell surface distribution of stem cell factor receptors. Exp Hematol 1995; in press.
47. Koller MR, Emerson SG, Palsson BO. Large scale expansion of human stem and progenitor cells in continuous perfusion cultures. Blood 1993; 82:378-84.
48. Steel M. Telomerase that shapes our ends. Lancet 1995; 345: 935-6.
49. Lansdorp PM. Telomere length and proliferation potential of hematopoietic stem cells. J Cell Science 1995; 108:1-6.
50. Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proc Natl Acad Sci USA 1994; 91:9857-60.
51. Niehuis AW, McDonagh KT, Bodine DM. Gene transfer into hematopoietic stem cells. Cancer 1991; 67:2700-4.
52. Moritz T, Keller DC, Williams DA. Human cord blood cells as targets for gene transfer: potential use in genetic therapies of severe combined immunodeficiency disease. J Exp Med 1993; 178:529-36.
53. Bertolini F, De Monte L, Corsini C, et al. Retrovirus-mediated transfer of the multidrug resistance gene into human hematopoietic progenitor cells. Br J Haematol 1994; 88:318-24.