

serve as a demonstration that cord blood cells can differentiate into non-hematopoietic tissues.

All children were prepared for transplant with myeloablative chemotherapy consisting of busulfan, cyclophosphamide and anti-thymocyte globulin. Prophylaxis against GvHD was administered with cyclosporine and methylprednisolone. Supportive care was provided with IVIG, G-CSF, low dose heparin for VOD prophylaxis, leukocyte depleted and irradiated PRBC and platelet transfusions, total parenteral nutrition, prophylactic antiviral and antifungal antibiotics and empiric antibiotic therapy for fever. Thirty five young children with Hurler syndrome (MPS I) were transplanted with partially HLA mismatched unrelated donor umbilical cord blood over the past 8 years. All had the severe phenotype. Neutrophil (ANC 500/ μ L) and platelet ($>50k/\mu$ L) engraftment occurred in a median of 20 and 63 days respectively. Moderate to severe acute GvHD occurred in 28 % of patients. Extensive chronic GvHD was not seen. Twelve percent of patients had serious events, graft rejection (n = 1), infectious deaths (n = 3), toxic death (n = 1, hyperammonemia). All other patients (87%) are surviving event-free for a median greater than 3 years. All surviving children remain full donor chimeras and have shown increasing velocities of gains of neurocognitive functions. Skeletal growth improved with only 4/11 children with severe kyphosis requiring orthopedic surgery post transplantation therapy. No child developed clinical cardiac disease and corneal clouding improved in all.

Additional children (60) with lysosomal storage diseases including metachromatic leukodystrophy, adrenoleukodystrophy and globoid leukodystrophy (Krabbe disease) have been transplanted with unrelated donor umbilical cord blood over the past 9 years. In asymptomatic children, disease was arrested before the onset of neurologic dysfunction. In symptomatic children disease progression was arrested within 6-9 months of the transplant procedure. In a child with advanced Krabbe disease who died 1 year post transplant, engraftment of donor cells was noted in the brain. Differentiation to oligodendrocytes was demonstrated in vitro and subsequently, in vivo. In a child with MPS III (Sanfilippo syndrome), donor cells differentiated into cardiac myocytes in the heart 6 months post transplant.

These studies suggest that cord blood is capable of transdifferentiation into non-hematopoietic lineages. Further studies are needed to fully define the potential of these cells for cellular therapies and tissue repair.

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UNRELATED DONOR CORD BLOOD TRANSPLANTATION FOR CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

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Cord blood (CB) contains a large amount of hematopoietic progenitors and over the last decade has been largely employed to transplant children with either malignant or non-malignant disorders. Advantages related to the use of CB cells are represented by the low risk of acute and chronic graft-versus-host disease (GVHD), prompt availability of this source of hematopoietic progenitors, which shortens the time needed to locate a suitable donor, and by the possibility of performing transplants in the presence of 1 or 2 HLA disparities in the donor/recipient pairs. Both these latter two factors can be relevant for treating children with malignancies, whose disease, often running an aggressive clinical course, might not allow an extended period of time for finding a suitable unrelated bone marrow donor. Hundreds of children with acute lymphoblastic or myeloid leukemia have received an allograft of unrelated CB cells. The experience derived from these patients has demonstrated that results achieved with this type of transplant are substantially comparable to those obtained in children given bone marrow transplantation. In particular, the risk of leukemia recurrence is not increased after CB transplantation. Disease status at time of transplantation is the main factor influencing patient's outcome, patients transplanted in 1st-2nd remission being those with the best results. Both a higher transplant-related mortality and an increased risk of leukemia recurrence contribute to the worse outcome of patients given the

allograft in more advanced disease. As most deaths occurring in children given CB transplantation are due to infectious complications (related to both delayed hematopoietic recovery and lack of adoptive transfer of memory T-cells) strategies able to accelerate both hematopoietic and immune reconstitution could widen the use of CB cells for transplantation.

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THE ROLE OF CORD BLOOD TRANSPLANTATION IN THALASSEMIA

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More than 20 years ago, the first successful cure of β -thalassemia major by bone marrow transplantation was reported. Since then, more than 1500 patients have been treated in this manner. In Pesaro Italy where more than 1000 patients have received marrow transplantation, the 20-year probability of thalassemia-free survival is approximately 70%. Updated results strongly suggest that improved outcomes across risk categories have occurred as effective supportive care and conditioning regimen modifications have been applied to this setting. As with other non-malignant conditions, the alternative of increasingly effective supportive care also impacts upon the decision to pursue transplantation, even when transplant outcomes are very good. Umbilical cord blood (UCB) transplantation, thus, must compare favorably to proven therapeutic alternatives before its use can be expanded. UCB is an attractive alternative to other stem cell sources due to its decreased incidence of graft-versus-host disease (GVHD) and rapid tempo of immunological reconstitution after transplantation. Initial results of UCB transplantation for thalassemia suggest that acceptable outcomes are possible if measures are taken to mitigate the risk of graft rejection. Among 44 patients with sickle cell disease or thalassemia who received augmented conditioning therapy, the event-free survival was 94% compared to 62% among those who received a standard combination of busulfan and cyclophosphamide, with or without horse anti-thymocyte globulin. These early results also support the importance of banking efforts to expand the collection of related and unrelated UCB units. The clinical experience of unrelated UCB transplantation for thalassemia remains very limited. New techniques to prevent GVHD and promote engraftment, coupled with refined donor selection criteria should expand the availability of transplantation for thalassemia major.

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RESULTS OF UNRELATED CORD BLOOD TRANSPLANT IN PATIENTS WITH BONE MARROW FAILURE SYNDROMES

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In Fanconi anemia (FA) patients without an HLA identical bone marrow donor, search for an unrelated cord blood donor is an option, however few results have been reported so far. In the Eurocord registry, we have analyzed 44 patients with FA receiving an unrelated cord blood transplant (UCBT). The median age was 7.7 years and median follow-up 20 months (8-83). At UCBT, the median number of neutrophils was 620/ mm^3 and 13 patients had received more than 20 red blood cell transfusions. The cord blood was HLA mismatched in 37 patients (HLA-A and B by serology and DRB1 high resolution typing, 5/6 = 21, 4/6 = 12 and 3/6 = 3). The median number of nucleated cells (NC) at freezing was $5.5 \times 10^7/\text{kg}$ and at infusion $4.7 \times 10^7/\text{kg}$. Eighteen patients received CY and irradiation based preparative regimens, 12 patients fludarabine (FLU) containing regimen, 6 CY alone and 8 other regimens. GVHD prophylaxis consisted of CSA and MTX (43%) or CsA alone (23%). Results: Neutrophil recovery at day 60 was 56% \pm 8%. Two factors were associated with higher probability of neutrophil recovery (cell dose and number of HLA disparities). Acute GVHD (II-IV) was 23% \pm 6% (grade II = 7%, III = 7%, IV = 11%) and chronic GVHD occurred in 4/21 patients at risk (19%). Two year survival was 36% \pm 7%. In univariate analysis factors associated with better survival were negative CMV serology, NC at freezing or infused, higher neutrophil count at UCBT and FLU containing regimen. In multivariate analysis only two

factors were associated with better survival: NC at freezing ($>5.5 \times 10^7/\text{kg}$) (HR = 0.25, $p = 0.004$) and FLU containing preparative regimen (HR = 0.16, $p = 0.018$). In patients receiving a higher cell dose, 2-y survival was 55% versus 16% for those receiving a lower dose ($p = 0.005$); it was 67% for those receiving a FLU containing regimen compared to 25% ($p = 0.016$) for those receiving other regimens. We conclude that results of UCBC for FA patients are acceptable and can be improved by better selection of the CB units and use of FLU in the preparative regimen.

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THE ROLE OF CD4⁺CD25⁺ T REGULATORY (T_{REG}) CELLS IN ALLOGENEIC BONE MARROW TRANSPLANTATION

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A subpopulation of CD4⁺ T cells that co-expresses CD25, the IL-2 receptor alpha chain, without evidence of prior activation, has been shown to be important in self-tolerance by suppressing autoimmune responses. These CD4⁺CD25⁺ Treg cells are present in rodents at a frequency of 8-12% of CD4⁺ T cells and in humans at lower frequencies (1-4%). In vitro, Treg cells are potent inhibitors of alloresponses. Depletion of either host CD25⁺ cells prior to BMT or donor CD25⁺ cells in the donor graft inoculum present markedly accelerated graft-versus-host disease (GVHD) lethality. Whereas supplementing the donor graft with fresh CD4⁺CD25⁺ Treg cells delayed GVHD lethality, adding ex vivo activated and expanded Treg cells virtually abolished GVHD lethality. These data are consistent with the fact that activated Treg cells are more potent suppressors than non-activated cells. In studies in which CD4⁺ effector T cells could be tracked in the whole animal, Treg cells were found to suppress effector T cell expansion or homing to lymphoid organs and GVHD target tissues. Tregs that co-expressed high levels of the lymph node homing receptor, L-selectin, were far more potent than L-selectin lo cells suggesting that homing of Tregs to secondary lymphoid organs is critical to preventing alloresponse initiation in vivo. In other studies, alloengraftment was markedly increased by the infusion of ex vivo activated and expanded donor Treg cells that expressed high levels of L-selectin. Such engraftment facilitation was not dependent upon the capacity of host T cells to receive TGF β signals. We conclude that the infusion of ex vivo activated and expanded CD4⁺CD25⁺ is a highly potent means of inhibiting GVHD and facilitating alloengraftment. Based upon the known effects of Tregs on suppressing GVHD, augmenting alloengraftment and immune recovery and preserving graft-versus-leukemia effects, it is clear that a clinical trial of CD4⁺25⁺ merits consideration in the context of allogeneic BMT.

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IMMUNE RECONSTITUTION FOLLOWING CORD BLOOD CELL TRANSPLANTATION

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Immune reconstitution after hematopoietic stem cell transplantation (HSCT) is a fundamental problem that affects the clinical outcome of transplantation, regardless of the stem cell source. Delays or defects in immune reconstitution contribute to susceptibility to infections with viruses, fungi, and encapsulated bacteria, EBV-lymphoproliferative disease, and possibly to relapse. Ultimately, immune reconstitution depends on generation of new T lymphocytes in the thymus, a process that depends on interactions of thymocytes with thymic epithelial cells (TEC). Critical for TEC support of thymopoiesis are the cytokines IL-7 and kit ligand (KL). Umbilical cord blood cell transplantation (UCBCT) is increasingly employed because of theoretical and empiric advantages such as ready availability and decreased risk of graft-versus-host disease

(GVHD). Some of the features of UCBC that make them attractive as a source of HSC may also contribute to difficulties in immune reconstitution. However, this is very difficult to analyze critically in clinical HSCT because of the large number of technical differences in transplant regimens, alloreactivity, and poorly understood variables such as donor and host variation in lymphopoiesis. One approach to understanding the outcome of UCBCT is based on analyzing the differences between the cellular components of UCBC vs other sources of stem cells. All stem cell sources contain mixtures of HSC, committed myeloid and lymphoid progenitors, and mature lymphocytes. Analyses of myeloid recovery have shown that HSC dose can be limiting, hence recommendations for a minimum number of phenotypic progenitor cells required for marrow recovery. Murine data from our laboratory suggests that thymic recovery may also be related to the dose of infused HSC or lymphoid progenitors. Although the number of HSC in UCBC sources may be limiting, the increased proliferative potential of cord blood HSC could also be advantageous. Besides reduced numbers of T lymphocytes in UCBC, a major difference between UCBC and other HSC sources, e.g., adult marrow or PBSC, is the nature of the mature T lymphocytes present in each product. The T lymphocytes in UCBC are predominantly naïve T lymphocytes while adults have mainly memory T lymphocytes. Naïve T lymphocytes require a greater amount of antigen to stimulate in vitro, compared to memory T lymphocytes. The decreased ability to stimulate naïve T cells may account for reduced alloreactivity in UCBC, but may also lead to decreased sensitivity to nominal antigens such as viruses. Thus, USCBC recipients may gain less protection from the adoptive transfer of mature T lymphocytes than BMT or PBSCT recipients. In addition, T lymphocytes from UCBC may have altered immune responses mediated by the exposure to immunomodulators in the placental microcirculation, e.g., G-CSF. Over the last five years, various studies have shown that the maintenance of mature T lymphocyte numbers in the periphery is mediated by homeostatic proliferation, in which dividing cells do not undergo activation or further maturation. The mechanism of homeostatic proliferation differs between cell types. Naïve T lymphocytes depend on self-antigen and IL-7 as signals for homeostatic proliferation. IL-7 therapy is an attractive strategy for promoting post-transplant thymopoiesis but may be complicated by exacerbation of GVHD. Recent murine experiments suggest that expansion of mature T lymphocytes by IL-7 may have a paradoxical inhibitory effect on thymopoiesis. Some approaches for translation of these experimental concepts into empirical studies in UCBCT will be described.

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INFECTIOUS DISEASE COMPLICATIONS AFTER UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN: IMPACT OF STEM CELL SOURCE

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Umbilical cord blood (UCB) is being increasingly used as an alternative source of hematopoietic stem cells (HSC) for unrelated donor (URD) transplantation. However, how infection risk after UCB transplantation (UCBT) compares with that seen after URD bone marrow (BM) transplantation (BMT) is not known. Therefore, we conducted a retrospective comparison of serious infectious complications in the first 2 years after HSC transplant in children transplanted for hematologic malignancy at the University of Minnesota. Our hypothesis was that due to HLA disparity, delayed neutrophil recovery, and the naivety of the neonatal immune system, UCBT recipients may be at a higher risk of both early and late infectious complications compared to recipients of unmanipulated BM. In this retrospective analysis, there were 136 children (<18 years of age) with a hematological malignancy who received cyclophosphamide 120 mg/kg and total body irradiation (TBI 1320-1375 cGy) followed by the transplantation of unmanipulated bone marrow (BM, n = 52), T cell depleted marrow (TCD, n = 24), or umbilical cord blood (n = 60). Overall, the cumulative incidence of one or more serious infections for the entire period was comparable between groups (BM 81%, TCD 83%, UCB 90%; $p = 0.12$). Analysis of infection density (episodes of serious infection/1000 patient days) within the time periods days 0-42, 43-100, and 101-