gressed to severe marrow hypoplasia prior to undergoing a 4/6 HLA matched UCBT at 7 months of age. She was platelet and packed red blood cell dependent as well as neutropenic. Prior to cord blood transplant she had a number of bacterial infections, and ultimately died on day +18 of overwhelming sepsis with Enterococcus gallinarum and extensive clotting of her descending aorta, kidneys and pulmonary vasculature. The second child also showed signs of thrombocytopenia immediately after birth, and received platelet transfusions prior to transplant. She had multiple infections with bacterial organisms. Her bone marrow showed minimal megakaryocytes with initially normal cellularity, however prior to UCBT her marrow cellularity started to decrease. Due to her sisters disease progression, she underwent a 6/6 HLA matched UCBT at 4 months of age. She engrafted on day +16. She had complications including and early Candidal bacteremia, a seizure and renal toxicity. She completely recovered and is currently more than one year post transplant, well engrafted with full donor chimerism and no evidence of GVHD. Following engraftment she has not had further infections and appears to be developmentally normal. Our two patients represent a variant of CAMT with radio-ulnar synostosis lacking the HOXA11 mutation. We speculate they may have had an immune deficiency as evidenced by their extensive infection history, which resolved after transplant in the surviving child. Unrelated donor cord blood transplant appears to be a curative option for these children, and proceeding to transplant earlier may improve outcome.

SOLID TUMORS

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AUTOLOGOUS STEM CELL TRANSPLANTATION FOR POOR PROGNOSIS GERM CELL TUMORS: LONG TERM FOLLOW-UP OF A MULTI-CENTER EXPERIENCE

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High dose chemotherapy followed by autologous stem cell rescue (autoSCT) has been used to provide long term disease control for patients with poor prognosis germ cell tumors (GCT). Seventy-one adult males from the Leukemia/BMT program of British Columbia (n = 54) and the Manitoba Blood and Marrow transplant program (n = 17) underwent autoSCT for GCT between 03/86 and 02/04. Median age at autoSCT was 31 years (range 16–58). Histological subtype included non-seminoma (n = 67) and pure seminoma (n = 4). Fourteen patients presented with primary extragonadal GCT involving mediastinum (n = 11), retroperitoneum (n = 1), and central nervous system (n = 2). Nine patients proceeded straight to autoSCT after a suboptimal PR to induction chemotherapy. The remaining 62 patients achieved acceptable responses to initial therapy only to have clear evidence of relapsed disease either by radiological or tumor marker progression. Disease status at the time of autoSCT was first partial remission (n = 15), recurrent chemosensitive disease (n = 51), recurrent chemoresistant disease (n = 4), and recurrent untested disease (n = 1). At the time of autoSCT, tumor makers were normal in 44 patients and elevated in 27 patients. High dose chemotherapy for patients with nonseminomatous histology was etoposide 3.0 g/m^2, carboplatin 0.8–1.2 g/m^2, and ifosfamide 6.0 g/m^2 or cyclophosphamide 7.2 g/m^2. The 4 patients with pure seminoma received cyclophosphamide 7.2 g/m^2 and carboplatin 1.5 g/m^2. Stem cell source was BM (n = 38), PB (n = 31), or both (n = 2). At a median follow-up of 7 years (range 1–18), 29 patients are alive and in remission. Thirty-one patients have relapsed post autoSCT. The 5 year EFS and OS for the entire 71 patients is 43% (95% CI 31-54%) and 44% (95% CI 32–55%), respectively. Extragonadal presentation was associated with a significantly reduced 5 year EFS (14% vs 50% $P = .001$) and OS (14% vs 51% $P = .007$). A completely normal tumor marker remission at the time of autoSCT was associated with a significantly higher 5 year EFS (63% vs 14% $P < .001$) and OS (63% vs 17% $P < .001$). The TRM at 5 years was 13% (95% CI 6%–22%). Four patients developed secondary malignancies post autoSCT. This retrospective review of a large number of patients with extended follow up demonstrates autoSCT to be an effective treatment for patients with relapsed GCT who can achieve tumor marker negativity. Extragondal presentations and failure to achieve a tumor marker negative remission pre-autoSCT are associated with extremely poor outcomes.

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NON-VIRAL TRANSFECTION OF IMMATURE DENDRITIC CELLS WITH GD_2 mRNA ELICITS AN ALLOGENEIC IMMUNE RESPONSE

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Vaccination with tumor antigen-loaded Dendritic cells (DC) has been shown to induce a CD4+ T helper cell (Th1) response. The purpose of this study is to develop a non-viral method of transfecting DC with mRNA encoding for GD_2 and measure the difference between autologous and allogeneic Th1 response. Immature DC were generated from CD14+ cells from human peripheral blood in RPMI media containing 10% fetal bovine serum, 1X pen-strep, granulocyte-macrophage stimulating factor (GM-CSF), and Interlukin-4 (IL-4). Immature DC were characterized by flow cytometry and were negative for CD14 and CD83, and positive for CD45, CD80, CD1a, CD86, HLA-DR, and CD11c. Full length human β1,4 N-acetylgalactosaminyltranserfase was cloned into a GFP expression vector and sequenced. GD_2 mRNA was transfected into immature DC at day 5 of culture using Qiagen Transmessenger. Transfection efficiency was determined by GFP fluorescence at 48 hours post-transfection via flow cytometry. DC transfected with water were used as a negative control. Cells were further matured with tumor necrosis factor α (TNF α) for an additional 24 hours post-transfection. T cells were plated in 24 well plates and co-cultured with autologous or allogeneic GD_2 mRNA transfected DC to prime the T cells. On day 8 of culture, the T cells were restimulated, and T cell response against antigen loaded DC, or water, was measured by the amount of IFN&gamm; secreted using Elispot. The number of spots in each well were counted and represent the amount of IFN&gamm; secreted by Th1 cells. Of the GFP-positive transfected cells, 33% (range 31.4–35.0) expressed GD_2 on the surface by flow cytometry. The amount and intensity of IFNγ secreted was significantly increased in mRNA transfected wells (mean of 31.13, range 3–60 spots) compared to water (mean of 16.38, range 3–36 spots). In addition the IFNγ secreted by individual T helper cells was significantly higher in the allogeneic transfected wells (mean of 47.5, range 30–57 spots) versus the autolgous wells (mean of 16.5, range 3–28 spots). These data demonstrate that we are able to transfect DC with GD_2 mRNA using a non-viral method and elicit an allogeneic immune response in vitro.

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GRAFT-VERSUS-BRAIN TUMOR EFFECT IN A CHILD WITH ANAPLASTIC ASTROCYTOMA AFTER CORD BLOOD TRANSPLANTATION FOR THERAPY-RELATED LEUKEMIA

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The graft-versus-tumor effect has been described for several solid tumors, but little is known regarding the susceptibility of brain tumors to allogeneic immune responses. We report a case of cord blood transplantation for therapy-related acute myelogenous leukemia in a 7-year old boy with active high-grade astrocytoma lesions. This represents the first reported case of a pediatric patient undergoing transplantation for therapy-related leukemia with a coexisting brain tumor. The patient received a

preparative regimen consisting of busulfan, cyclophosphamide, and anti-thymocyte globulin for a 5 out of 6 HLA-antigen-matched unrelated donor cord blood transplant. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methylprednisolone, and the patient experienced isolated acute skin GVHD. Notably, several astrocytoma lesions regressed or decreased in size between 3 and 8 months after transplantation, associated with a lymphocytic CSF pleiocytosis with elevated CSF protein. The patient remains leukemia-free with stable brain lesions 25 months after transplantation. This case illustrates the potential for donor-derived immune cells to control brain tumors, and suggests that allogeneic bone marrow transplantation may represent an important immunotherapeutic strategy for the aggressive treatment of poor-prognosis brain tumors. Future clinical protocols should seek to further characterize the nature of the immune response to brain tumors after allogeneic bone marrow transplantation.

STEM CELL BIOLOGY

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USE OF CHROMATIN MODIFYING AGENTS FOR EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD STEM CELLS

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The fixed number of hematopoietic stem cells (HSCs) within a single cord blood (CB) unit has limited the use of CB for allogeneic transplantation in adults. Efforts to promote self-renewal and expansion of HSCs have been met with limited success. Using presently available ex vivo culture techniques, HSCs lose their functional properties in proportion to the number of cellular divisions they have undergone. We hypothesized that chromatin modifying agents, 5-aza-2′-deoxycytidine (5azaD) and histone deacetylase inhibitor, trichostatin A (TSA) could reactivate pivotal genes required for retaining the functional properties of dividing HSC to permit transplantation of adults. A 12.5-fold expansion was observed in the 5azaD/TSA treated CD34+CD90+ cell cultures containing SCF, thrombopoietin, and FLT3 ligand (cytokines) in comparison to the input cell number. Despite 9 days of culture, 35.4% ± 5.8% (n = 10) of the total cells in the cultures exposed to chromatin modifying agents were CD34+ CD90+ compared to 1.40% ± 0.32% in the culture containing cytokines alone. The CD34+CD90+ cells were associated with a 9.8-fold increase in the numbers of CFU-mix and 11.5-fold expansion of cobblestone area-forming cells (CAFC). The frequency of SCID repopulating cells (SRC) was 1 in 26,537 in primary CB CD34+CD90+ cells but was increased to 1 in 2745 CD34+CD90+ cells following 9 days of culture in the presence of 5azaD/TSA resulting in a 9.6-fold expansion of the number of SRC. In contrast, the cultures lacking 5azaD/TSA had a net loss of both CFC/CAFC as well as SRC. The expansion of cells maintaining CD34+CD90+ phenotype was not due to the retention of a quiescent population of cells since all of the CD34+CD90+ cells in the culture had undergone cellular division. CD34+CD90+ cells that had undergone 5–10 cellular divisions in the presence of 5azaD/TSA but not in the absence still retained the ability to repopulate NOD/SCID mice. We next assessed the effect of 5azaD/TSA treatment on the expression of *HOX-B4*, a transcription factor which has been implicated in HSC self-renewal. A significantly higher level of *HOXB4* protein was detected by western blot analysis after 9 days of culture in the cells treated with 5azaD/TSA as compared to cells exposed to cytokines alone. The almost 10-fold increase in SRC achieved using the chromatin modifying agents may be sufficient to increase the numbers of engraftable HSC within a single human CB unit for adult recipients.

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ADULT HUMAN HEMATOPOIETIC CELLS DIFFERENTIATE INTO MATURE T CELLS VIA A CD3-4+8- INTERMEDIATE WITHIN THE MOUSE THYMIC MICROENVIRONMENT; A NEW MODEL SYSTEM FOR THE STUDY OF HUMAN THYMOCYTE DEVELOPMENT FURTHER ENHANCED BY ANTI-MURINE c-Kit mAB

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Normal T cell differentiation occurs within the thymus. We previously found that human thymocyte precursors develop via a novel CD3-4+8- intermediate population, utilizing SCID-hu thymic grafts (Kraft,Weissman,Waller;JEM). We have recently worked to develop a more convenient robust model of human hematopoiesis utilizing RAG2/Common Gamma Chain double KO mice transplanted with hematopoietic progenitor cells from adult human donors, and hypothesized that human engraftment would be enhanced by selective inhibition of murine hematopoisis by administration of anti-murine c-Kit mAb. **Methods:** Mobilized CD34+ cells from healthy adults were obtained to >90% purity by CliniMacs. 2–8 × 10^5 CD34+ cells were injected intrahepatically into newborn RAG2 DKO pups following 4Gy of irradiation. At serial time points, human CD45+ chimerism was measured within the recipient marrow, blood, spleen, liver, lymph node, and thymus. A subset of recipients were treated with the anti-murine c-Kit mAb (ACK2) starting at D +14. **Results:** Human engraftment was detectable in CD34+ transplanted mice, and this was enhanced by suppression of murine hematopoiesis by anti c-Kit mAb. Robust human thymopoeisis was observed. A mean of 63% of cells within the thymus were human derived. At earlier time points (4–6 weeks post transplant) the recipient thymus were found to contain high fractions of CD45+CD3−4−8− cells (making up 30–40% of human cells within the thymus) and the CD3−4+8− (20–30%) and CD4+8+ (30–70%) intermediate populations with very rare mature CD3+4+8− or CD3+4−8+ T cells. At later time points the fraction of immature CD3−4−8− and CD3−4+8− populations declined and increasing populations of mature CD3+4+8− and CD3+4-8+ populations were identified in distributions similar to a normal thymus. **Conclusions:** Human T cell development appears to progress normally within a murine thymic microenvironment. The early development of a CD3−4+8− intermediate suggests that T cell development occurs via this population, unlike the CD3−4−8+ intermediate found in mice thymopoesis, suggesting that the pathway of human T cell differentiation is intrinisic to the human thymocytes, and is independent of whether the thymic stroma is human or murine. This robust model system enabling study of human thymopoesis utilizing hematopoietic stem cells from normal and diseased adults human donors may provide significant advantages for the study of human intrathymic T cell differentiation and function in vivo.

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$CD8^+/TCR^-$ GRAFT FACILITATING CELLS ENHANCE HSC FUNCTION VIA INDUCTION OF LOW LEVELS OF TNF-α

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Approaches to enhance engraftment of HSC when cell numbers are limiting remain an elusive goal in bone marrow transplantation. We recently reported that $CD8^+/TCR^-$ facilitating cells (FC), a subpopulation of BM cells containing predominantly $B220^+/CD11c^+/CD11b^-$ tolerogenic precursor-plasmacytoid dendritic cells, enhance HSC engraftment in allogeneic recipients. FC are themselves tolerogenic, directly preventing GVHD. Additionally, FC significantly enhance engraftment of limiting numbers of HSC in syngeneic recipients. In the present studies, we investigated the mechanism of FC function. Here we show for the first time that FC significantly increase HSC