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

clonogenicity in vitro via production of inducible TNF-α upon contact with HSC. Co-culture of FC with HSC induces production of physiologically relevant low levels of TNF-α by FC. FC from TNF-α-deleted (TNF-$\alpha^{-/-}$) mice are significantly impaired in function in vitro and in facilitating HSC engraftment in vivo in syngeneic recipients as well as allogeneic recipients. A syngeneic model for limiting numbers of HSC (c-Kit^+/$Sca\text{-}1^+$/Lin^-) was used to evaluate facilitation by TNF-$\alpha^{-/-}$ FC. Notably, while 80% of recipients of 500 B6 HSC + 30,000 B6 FC engraft durably, none of the recipients of 500 B6 HSC plus 30,000 TNF-$\alpha^{-/-}$ FC engrafted. Furthermore, neutralization of TNF-α on FC using anti-TNF antibody results in loss of FC effect on HSC clonogenicity in vitro, confirming a direct and critical role for TNF-α in FC function. Notably, co-culture of FC with HSC is associated with significant upregulation of the anti-apoptotic I-κB family member Bcl-3 in HSC. Blocking of TNF-α on FC abrogates the upregulation of Bcl-3 in HSC. Taken together, these findings demonstrate that TNF-α-induced in FC by contact with HSC affects highly primitive HSC and identify Bcl-3 as a possible pathway for TNF-α in promoting HSC survival and engraftment efficiency.

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SUPERIOR EX VIVO CORD BLOOD TNC AND HEMATOPOIETIC PROGENITOR CELL EXPANSION FOLLOWING CO-CULTURE WITH BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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One factor limiting the therapeutic efficacy of cord blood (CB) hematopoietic progenitor cell (HPC) transplantation is the low cell dose of the graft. Low cell dose is associated with an increased incidence of delayed or failed engraftment. However, cell dose can be increased and the efficacy of CB transplantation potentially improved by ex vivo CB expansion prior to transplantation. Two ex vivo CB expansion techniques were compared and hematopoietic output followed by measuring total nucleated cell (TNC), $CD133^+$ and $CD34^+$, colony-forming unit (CFU) and cobblestone area-forming cell (CAFC) numbers. **Technique 1:** Ex vivo culture of $CD133^+$-selected cells; and **Technique 2:** Co-culture of unmanipulated CB with allogeneic bone-marrow-derived mesenchymal stem cell (MSC) monolayers. In both cases, ex vivo culture was performed in medium supplemented with granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF) and either thrombopoietin (TPO) or megakaryocyte growth and differentiation factor (MDGF). When compared to ex vivo liquid culture, ex vivo CB-MSC co-culture (1) required less cell manipulation resulting in less initial HPC loss and (2) markedly improved TNC (>10-fold), CFU (>25-fold), $CD133^+$ (>7-fold) $CD34^+$ (>14-fold), $CAFC_{wk2}$ (>200-fold), and $CAFC_{wk6}$ (>44-fold) output. In addition, MSC have also been shown to promote hematopoietic engraftment in animal models and to possess immunomodulatory activities and have been shown to reduce the severity of graft versus host disease. One concern associated with any ex vivo expansion strategy is that short-term reconstituting, lower quality HPC will be expanded at the expense of long-term reconstituting, higher quality HPC, thereby significantly impacting the hematopoietic reserve of the graft. Here we demonstrate that although neither technique significantly expanded the more primitive $CAFC_{wk6}$ population, they were better preserved in the CB-MSC co-culture system. Although the expansion of the more mature components of the hematopoietic system (TNC, CFU, $CD133^+$, $CD34^+$, and $CAFC_{wk2}$) in both cases was at the expense of the more primitive HPC, in neither case was the $CAFC_{wk6}$ compartment completely exhausted, thereby potentially preserving part of the hematopoietic reserve of the graft. In improving TNC and HPC expansion, ex vivo CB-MSC co-culture therefore holds promise for improving engraftment kinetics and post-transplant complications in CB transplant recipients.

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IDIOPATHIC SECONDARY POST-TRANSPLANT THROMBOCYTOPENIA (ISPT) FOLLOWING AUTOLOGOUS HEMATOPOIETIC PROGENITOR CELL TRANSPLANTATION (HPCT) IS ASSOCIATED WITH TRANSPLANTATION OF LOWER NUMBERS OF PRIMITIVE HEMATOPOIETIC PROGENITORS

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Background: Platelet counts often drop after initial hematopoietic engraftment. We hypothesized that secondary thrombocytopenia is related to the stem cell content of the graft. **Methods:** We performed a retrospective analysis of 323 consecutive patients who underwent autologous blood (97%) or BM (3%) HPCT between 2000 and 2005 for Hodgkin's lymphoma (n = 53), NHL (n = 85), multiple myeloma (n = 166), acute leukemia (n = 16), and solid tumor (n = 5). ISPT was defined as a >50% decline in blood platelets following initial engraftment to a value less than 100 K/mcL, in the absence of infection or relapse, in the first 100 days post-transplant. Engraftment, transfusions, infections, relapse, and survival were recorded with a median follow-up of 410 days. **Results:** 303 patients were evaluable and achieved a transfusion-independent platelet count of >20K/mcL, at a median of 17 days post-transplant. The maximal platelet count was a median of 192 K/mcL and occurred on a median of 22 days. Sixty-three patients (21%) had secondary thrombocytopenia after initial engraftment, with 3 cases of relapse and 11 cases of sepsis, leaving 49 patients (15%) with ISPT, and 254 controls. Patients with ISPT engrafted at a median of 17 days, with a maximal platelet count of 162 K/mcL on day 19. Platelet counts in ISPT patients dropped to a nadir of 34 K/mCL (range 4 K–98 K/mcL) on day 35 with subsequent recovery to a median of 148 K/mcL on day 71. Mean CD34+ cells/kg transplanted in ISPT patients ($9 \pm 7 \times 10E6$/kg) were similar to cell doses transplanted in controls ($11 \pm 14 \times 10E6$/kg), but IPST patients received fewer CD34+CD38− HPC ($0.08 \pm 0.08 \times 10E6$/kg) versus controls ($0.13 \pm 0.24 \times 10E6$/kg, $P = .005$, 2 sided T-test). HIT and anti-platelet antibodies were absent, and only 16% of BM biopsies in patients with ISPT had increased megakaryocytes. Fourteen of forty-nine patients with ISPT received steroids and/or IVIG and had similar platelet recoveries to those not treated. Lymphoma patients and recipients of busulfan conditioning regimens were over-represented in patients with ISPT compared to controls ($P < .001$). Three-year actuarial survival was not significantly different between the 49 ISPT cases (79%) versus the 254 controls (76%). **Conclusions:** ISPT occurring within the first 100 days after autologous HPCT is common, and associated with transplantation of lower numbers of phenotypically primitive HPC. IPST does not appear to be an auto-immune phenomenon, is self-limited, and is not associated with adverse long-term survival (Table1).

Table 1. *Comparison of IPST Cases vs Controls*

	Age (years)	Gender (male)	HL and NHL*	Pre-trx XRT	Median day Plt engrafted	BM cellularity day 40–45	Platelet transfusion increment
IPST N = 49	**48 years**	**55%**	**73%**	**4%**	**17 days**	**35%**	**32K/mcL**
Controls N = 254	**51 years**	**63%**	**36%**	**7%**	**17.5 days**	**34%**	**31K/mcL**

*$P < .001$