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NON-MYELOABLATIVE STEM CELL TRANSPLANTATION IN NEWLY ESTABLISHED DONOR STROMAL MICROENVIRONMENT FOR FACILITATION OF HEMATOPOIETIC RECONSTITUTION

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Following allogeneic stem cell transplantation (SCT) migrate to the hematopoietic microenvironment in bones, but the stroma remains of host origin. We hypothesized that replacement of host with donor stromal microenvironment supporting and regulating hematopoiesis may enhance engraftment and differentiation of bone marrow cells (BMC), especially in patients with myelofibrosis. Our earlier work suggested that SCT with donor stromal microenvironment modifies GVHD. We have recently documented that transplantation of BMC mixed with demineralized bone matrix (DBM) may be sufficient for formation of new bone and hematopoietic mircoenvironment. Tested in rats, implantation of DBM/BMC composite intraosseously or under the kidney capsule resulted in new bone formation containing donor hematopoietic tissue. Based on the murine data, we introduced the use of intra-osseous transplantation of DBM/BMC for patients with existing or anticipated marrow failure, such as myelofibrosis, following non-myeloablative conditioning (NST). Our 1st patient was 54 yrs old woman with RAEB-T and myeloid fibrosis, with no hematologic reconstitution following NST (fludarabine, busulfex & ATG) until week +4. Intraosseous (femora and pelvis) transplantation of DBM/BMC was accomplished with no further conditioning from the same donor, assuming the problem was engraftment failure rather than rejection. Early 3-lineage reconstitution of donor (male) cells was confirmed within one week. Currently, the patient is more than 2 years out with mild chronic GVHD, with 100 percent normal male karyotype. Bone biopsy, laser capture microdissection and PCR revealed 100 percent donor-derived blood cells, stroma and bone cells. Two additional patients underwent successful intra-osseous DBM/BMC transplantation into one femur following NST, with fast 3-lineage engraftment. Procedures were uneventful. All patients are disease free, with 100 percent donor type cells. Considering our experimental and clinical experience, intra-osseous transplantation of DBM/BMC may provide a new tool for enhancing allogeneic hematopoietic reconstitution, especially for patients with myelofibrosis.

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ISOLATION OF OLIGODENDROCYTE PRECURSORS FROM UMBILICAL CORD BLOOD

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Introduction: The treatment of childhood leukodystrophies with umbilical cord blood (UCB) transplantation has resulted in cognitive and functional stabilization, but with minimal improvement in neurologic and motor deficits preexisting at the time of transplant. The lack of improvement in motor function may be due to the irreversible damage to motor neurons before transplantation. Conversely, trafficking of transplanted cells may not occur for months after transplant, missing a potential therapeutic window. The delivery of cells directly to the brain may hasten engraftment in and repair of neurologic tissue, facilitating clinical improvement in these patients. We hypothesized that UCB contains cells capable of differentiating into precursors of some neural lineages, specifically oligodendrocytes, which could eventually be used for transplantation in patients with these demyelinating or dismyelinating diseases. To this end, we attempted to culture neural progenitor cells from UCB ex vivo. **Methods:** Mononuclear cells were isolated from fresh umbilical cord blood units with hespan and ficoll density gradient separation, and cultured in flasks and LabTek® chamber slides at a concentration of 3-4 $\times$ 10^6 cells/mL in media containing platelet-derived growth factor (PDGF) (5ng/mL), neurotrophin-3 (1ng/mL), tri-iodothyronine (30ng/mL), vascular endothelial growth factor (VEGF) (10ng/mL), and 10% fetal calf serum. An adherent cell population was observed after one week of culture. Adherent cells grown on LabTek® slides were fixed with 4% paraformaldehyde, stained and scored using immunocytochemical techniques for the hematopoietic markers CD45 and CD34, human leukocyte antigen (HLA) class I and II, microglial markers CD68 and CD11b, the astrocyte marker GFAP, oligodendrocyte markers O1, O4, and myelin basic protein (MBP), and neuronal markers NeuN and β-tubulin III. **Results:** Approximately 60% of the isolated adherent cells appeared long and spindle-like, many with branches extending from the cell body. These cells displayed positive fluorescent staining for CD45, O1, O4, MBP, HLA I, and HLA II. The cells did not express CD34, CD68, CD11b, GFAP, NeuN, or β-tubulin III. **Conclusions:** The pattern of staining observed in this UCB-derived cell population is suggestive of oligodendrocyte differentiation. These cells may have the potential for therapeutic trials in patients with leukodystrophies.

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A REQUIREMENT FOR THE SPLEEN IN PREVENTING SUSTAINED IMBALANCE OF B220+ CELLS AND GR-1+ CELLS DURING RECONSTITUTION FOLLOWING SYNGENEIC AND ALLOGENEIC BMT

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The murine spleen is an active hematopoietic compartment following BMT and a target for preferred engraftment by a subset (Rho^{hi}) of hematopoietic stem cells (HSC). To determine the contribution of the spleen to hematopoietic reconstitution after BMT, the relative pattern of donor cell reconstitution and progenitor cell (PC) distribution was examined in splenectomized (Splx) recipients. Two weeks post-BMT, removal of the spleen results in significant increases in marrow PC activity compared to sham-Splx controls in syngeneic and allogeneic BMT. Peripheral and BM distribution of donor cells representative of lymphoid (B220+) and myeloid (Mac-1/Gr-1+) cells was then assessed to >3 months post-BMT. A significant ($p < 0.05$) elevation of circulating B220+ cells was present in Splx recipients from 14 to 90 dys post-BMT, declining to sham control levels by >100 dys post-BMT. In contrast, the % of circulating granulocytes (Gr-1+/Mac-1+) was significantly lower in Splx compared to sham controls to >100 dys post-BMT. Three months post-BMT, the marrow compartment in Splx recipients demonstrated >3x ($p < 0.0001$) increases in sIgM+D+ cells, although differences in circulating B220+ cells among the two groups had then diminished by this time point, indicating a preferential accumulation of sIgM+D+ cells in this compartment. Initial results examining LPS-stimulated PBMC proliferation are consistent with elevated levels of circulating B220+ cells (<100 dys), indicating the B cells are functional in Splx recipients. Additionally, consistent with elevated mature "recirculating" B cells in the marrow compartment, LPS-induced proliferation levels were significantly higher ($p < 0.05$) in these Splx recipients at >100 dys post-BMT. The *in vitro* response of BM cells to IL-7 in Splx recipients was significantly ($p < 0.05$) elevated, suggesting that the effects of the spleen on B220+ cells may be "upstream" of marrow sIgM+D+ cells. A decline in BM cell proliferation in response to IL-3 also occurred, thus paralleling the observed peripheral decline in mature myeloid cell subsets. These data indicate that the absence of the spleen in BMT recipients could prompt sustained imbalance in the relative distribution of defined populations of the lymphoid and myeloid lineages. Thus the splenic compartment is important in BMT both as a site of hematopoiesis and in the regulation of defined peripheral populations of the lymphoid/myeloid lineage.

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COMPARISONS OF HEMATOPOIETIC SUPPORT BY ADIPOSE-DERIVED AND BONE MARROW-DERIVED STROMA

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Adipose-derived stromal (AdS) cells are multipotent mesodermal progenitors with developmental potentials similar to those ascribed