isopicnic Fraction IV (1.090 g/ml) separated on gradient of percoll. As it turned out both induced NS activities were discovered in this Fraction IV. In this work we were successful to obtain two separate cell fractions with floating density of 1.080 and 1.090 g/ml. The former fraction strictly showed only NS1 activity but the latter one showed only NS2 activity. The role of every NS subpopulations in different immunological processes has to be clarified.

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IMMUNOTHERAPY AND SINGLE AGENT CHEMOTHERAPY IN METASTATIC BREAST CANCER

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In metastatic breast cancer there is a high response rate greater than 50% after high dose (HD) chemotherapy with autologous stem cell support in chemotherapy sensitive disease. However, the median duration of response is limited to 18 months. We have treated patients with multiple chemotherapy regimens after HD and demonstrated an increase in the median progression free survival to 42 months. We have studied the role of immunotherapy added to a single agent chemotherapy program in thirteen patients predominantly with minimal residual disease as demonstrated with our cytokeratin analysis in the bone marrow. The immunotherapy program consisted of GM-CSF 250 micrograms subcutaneous daily for three days followed by 1.5 million International units of Interleukin-2/m2 subcutaneous daily for ten days. Treatment was initiated after patients failed to respond to single agent chemotherapy. Treatments were given on a monthly basis. Of the thirteen patients there were nine responders. The median duration was 14 months. Seven of the nine patients are still responding. Interestingly, the nine responders are all Her-2 positive and treated with Herceptin on a weekly basis, also during immunotherapy treatment. Toxicity is minimal and this treatment does not interfere with quality of life.

STEM CELL BIOLOGY

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REPAIR OF INFARCTED HEART BY EMBRYONIC STEM CELLS

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The objective of this work is to determine the efficacy and safety of Embryonic Stem (ES) cell therapy in murine heart infarct model as well as to establish functional improvement in the heart function following cellular therapy. Methods: 12 C57Bl/6J female mice (3 months old) underwent left coronary ligation under general anesthesia with isoflorane. Enhanced yellow fluorescent protein (EGFP) marked murine ES cells (R1/129) and ES-CCE Cell without marker fluorescence protein were injected into the border area of the infarcted zone of murine heart. Serial frozen sections of hearts were made at 24 hours, 1 and 2 weeks post injection. EGFP staining was double detected using Zeiss epifluorescent microscope and determined by immunohistochemistry using anti-GFP antibody. GFP, MEF2 and GATA-4 protein expression were detected using western blot assay by anti rabbit GFP, MEF2 and GATA-4 antibody. Cardiac function was determined by echocardiography and exercise testing using motor-driven treadmill. Results: Initial operative survival of infarcted mice was 100%. Zeiss epifluorescent microscope showed GFP signal in infarcted cardiac area in mice injected with EGFP-ES and not in ES-CCE injected mice. Bright field microscopy of the same sections showed similar results using anti-GFP immunostaining. Western blot showed that GFP protein was detectable in ES-injected infarcted mice after 2 weeks but not in ES-CCE injected mice, however, MEF-2 and GATA-4 proteins were detectable in both ES cells injected mice. Echo performed at one week after infarction revealed large infarcts in all surviving infarcted animals. Exercise treadmill (ETT) performed 1 week after infarction showed 80% reduction in exercise tolerance compared to sham controls (same operation but no coronary ligation) and un-operated controls. Conclusions: 1-Implantation of ES cell into the infarcted region is feasible by direct injection into the periphery of the infarcted zone. 2-With new anesthesia and surgical skills, we are able to generate large infarcts in mice that lead to reduction in cardiac function easily assessed by echo and ETT. 3-Further studies are underway to assess the role of ES cell therapy on cardiac function and remodeling.

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RECONSTITUTION OF VASCULAR ENDOTHELIUM FOLLOWING TRANSPLANTATION OF BONE MARROW DERIVED STEM CELLS

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Bone marrow derived (BM) cells have recently been shown to participate in angiogenesis following ischemic injury. It remains unknown whether the vascular damage associated with lethal does of irradiation provides the signals necessary to induce BM cells to differentiate into endothelium. To address this question, 5E6 unfractionated BM cells from male C57BL/6-EGFP mice were transplanted into lethally irradiated (1200 cGy) C57BL/6 female recipients. Examination of the liver of recipient mice at 5 days post transplant revealed only rare GFP+ cells. However, by day 14, GFP+ donor cells were readily detected within the liver including the portal veins (PV), central veins, hepatic arteries and the microvasculature. The presence of donor derived cells within the intima of the vascular tissue was confirmed by in situ hybridization using a Y chromosome specific probe. A mean of 38% of the PV demonstrated > 3 GFP+ cells incorporated into the intima of the vessel wall (~10% of luminal cells per cross section). By 6 months post transplant, >50% of the PV contained GFP+ cells. Luminal GFP+ cells evaluated by confocal microscopy co-expressed both CD31 and von Willebrand factor (vWF) but did not express CD45. A similar donor derived endothelial phenotype was found in vasculature of the lungs and the gut. To determine if donor derived endothelial cells could be generated from the HSC compartment, c-kit + Sca-1 + Lin- (KSL) cells were sorted from the BM of adult GFP mice and transplanted into irradiated recipients. Evaluation of the liver of these chimeric recipients showed donor derived GFP+ CD31+ vWF+ CD45- cells in a dose dependent manner with a frequency similar to that observed following the transplantation of an equivalent dose of unfractionated BM. Donor derived endothelial cells were also readily detected in the lungs and the gut. These results indicate that KSL cells can be induced to give rise to multilineage hematopoiesis and contribute extensively to vascular endothelium in the irradiated transplant recipient.

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UMBILICAL CORD BLOOD CELLS ENGRAFT AND DIFFERENTIATE IN NEURAL TISSUES AFTER HUMAN TRANSPLANTATION

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Bone marrow transplantation can prevent progression of neurologic damage caused in infants and young children with inborn errors of metabolism if the transplant procedure is performed before extensive neurologic damage has occurred. It has previously been shown that donor derived hematopoietic cells migrate into brain to deliver enzyme to neighboring host, enzyme deficient cells. However, differentiation of marrow derived donor cells into cells of neural or glial origin has not been definitively demonstrated. Over the past 6 years, 20 pts with Krabbe disease (globoid leukodystrophy) have been transplanted at Duke Medical Center with unrelated umbilical cord blood (UCB). Five pts, transplanted as neonates have derived significant benefit from the procedure with increasing neurocognitive development, correction of nerve

conduction abnormalities and increasing myelination on MRI scans. Pts transplanted later in the course of the disease have had less dramatic but measurable improvements, particularly in language and cognitive skills. We hypothesized that UCB-derived cells migrated to brain and differentiated into non-hematopoietic cells in these pts but could not study this further with non-invasive techniques. Unfortunately, one baby with advanced Krabbe disease, transplanted with a UCB donor of the opposite sex at 8 months of age, died 1 yr post transplant. Her brain was studied to determine whether donor derived cells were present at the time of her death. Brain tissue was fixed in formalin, sectioned and stained with histochemical stains for glial and neuronal tissues and counterstained with FISH for the XY chromosomes to differentiate donor and host cells. We found extensive distribution of host cells in blood vessels, peri-ventricular tissues, white matter of the cerebral cortex, cerebellum, choroid plexis and forebrain parenchyma. Differentiation of donor cells to microglia and choriod plexus cells was present, but differentiation into cells of neuroectodermal origin (e.g neurons, astrocytes, or oligodendrocytes) was not found. These results demonstrate that donor-derived UCB cells can extensively distribute in brain tissues after UCB transplantation. Transdifferentiation across germ cell layers was not demonstrated.

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EMBRYONIC STEM CELLS SURVIVE AND PROLIFERATE AFTER INTRA-PERITONEAL IN UTERO TRANSPLANTATION AND PRODUCE TERATOCARCINOMAS

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Embryonic stem cells (ESC) support murine fetal development if injected in blastocysts and can engraft in conditioned newborns. However, little is known about their behavior after the in utero transplantation. The aim of this research is to establish a model for study of the in vivo differentiation of ESCs after the in utero transplantation. We hypothesize that the in utero transplanted ESC integrate into the fetus and, based on their pluripotency, may represent a therapeutic alternative for prenatally diagnosed diseases. ESC genetically engineered to express yellow fluorescent protein (YFP-ESC) were transplanted intra-peritoneally in utero at E14-15. The presence of YFP signal was analyzed in various tissue of the viable offsprings at different ages (4-8 weeks) and was quantified by digitalized fluorescence microscopy on analysis of tissue supernatants. The YFP-ES-derived cells were found only in the liver without any evidence of YFP signal in other organs. Extensive peritoneal teratocarcinomas with supradiaphragmatic involvement was generated after the ESC in in utero transplantation in allogeneic mice. Also, clinical and histopathological pictures suggestive of graft-versus -host disease were present in two out of five haploidentical mice. Studies that address the issue of ESC differentiation into hepatic cells within this model are in process and will be presented. The promise and the potential risks of the ESC transplantation have to be carefully considered.

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BRAIN FROM BLOOD IN HUMANS

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Adult hematopoietic stem cells (HSC) have the capacity to self-renew and differentiate into all hematopoietic lineages. Recent studies in humans have found that bone marrow derived stem cells can function as regenerative progenitors for the liver, kidney, heart, musculoskeletal tissue, and gastrointestinal tract. In the brain, animal studies have found that murine HSC can differentiate into neurons of the adult mouse. Following these reports, we investigated whether human HSC contribute to adult human neurogenesis. Autopsy brain specimens from female recipients of therapeutic HSC transplantation from male donors where analyzed for cells containing Y chromosome. In these cases, hippocampal cells containing Y chromosome were found. Most Y-positive cells were non-neuronal, with transgender neurons (beta-3-tubulin positive) comprising only 1% of total neurons. In addition, these Y-chromosome neurons were not a product of fusion, as evidenced by presence of only one X chromosome. Our findings demonstrate that postnatal human neuropoiesis is present and that human hematopoietic cells have the capability to generate neurons, albeit at a very low level. The biologic implications suggest that the HSC or a hematopoietic progenitor responds to instructive neurotrophic cues, crosses the blood-brain-barrier, diffuses into central nervous system tissues and activates previously dormant neuron-specific genetic programming. Together these observations challenge the restricted notion of unidirectional ontogenic maturation and uncover a mounting HSC plasticity repertoire. Our findings also suggest that human HSC may serve as a therapeutic source for regenerative neuropoiesis.

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AUTOLOGOUS STEM CELL COLLECTION IN POLYCYTHEMIA VERA

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Allogeneic Tx can eradicate MF. We reported resolution of MF after ablative syngeneic Tx in a patient with spent phase PV (B J Haematol 117:246). Hence GvL is not required to eliminate MF. Ablation with ASCT could represent a major advance for spent phase PV. In PV, the optimal timing and the influence of organomegely, myelosuppression and MF on autologous collection are uknown. Between 08/94 and 01/02, 16 pts with PV underwent stem cell collection. Mobilization was G-CSF 10 mcg/kg x 5d. Minimum target was 2.5 X 10^6 CD34+/kg. All myelosuppression but anagrelide was stopped a minimum of 2 wks before collection. M:F ratio was 7/9. Median ages at Dx and collection were 47 and 57. Organomegaly was present in 10 pts (63%) and moderate or extensive MF in 4 pts (25%). Seven pts were receiving myelosuppression. Three pts had clonal cytogenetic abnormalities. For the whole cohort median TNC and CD34+ counts were 8.3 X 10^8/kg and 4.98 X 10^8/kg. No organomegaly predicted for higher TNC and lower CD34+ contents but differences were NS (p=0.16 and 0.1). MF adversely affected TNC (p=0.05) but not CD34+ (p=0.8). Time from Dx and myelosuppresion had no influence on TNC and CD34+ . One pt had CD34+ below target (2.2 X 10^6/kg). See table for details. Autologous collection of peripheral blood stem cells is feasible in PV pts several years after DX. Organomegaly and MF are not contraindications for collection. Myelosuppresion up to 2 weeks prior to mobilization appears safe in terms of cell contents. Studies are now required to determine the safety and efficacy of ablation and ASCT to reestablish effective hematoposiesis in spent PV.

	TNC e8/kg		CD34+ e6/kg	
	N	Y	N	Y
Organomegaly	12.2	6.6	3.06	8.30
T from Dx>10y	0.7	8.7	2.9	11.9
MF	10.3	3.8	6.09	3.25
Myelosuppression	8.4	9.1	3.7	9.8

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PREFERENTIAL EXPANSION OF CORD BLOOD EARLY PROGENITORS ENABLED BY LINEAR POLYAMINE COPPER CHELATORS

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We demonstrated that the polyamine copper chelator, tetraethylenepentamine (TEPA), extended the long term cord blood CD34+ cultures (Peled et al, Brit. J. Haematol. 116:655 2002).To study the effect of TEPA on long-term expansion, we adopted a