STEM CELLS

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Plenary Sessions *Page*

Poster Sessions

Hematopoietic Stem Cells

H 001 DEFINING POPULATIONS OF MULTILINEAGE HEMOPOIETIC STEM CELLS
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PHSC (Primitive Hemopoietic Stem Cells) continuously repopulate all lymphoid and myeloid systems from fetal life through old age. They are vital in every treatment requiring long term production of lymphoid or myeloid cells, such as gene transplantation via autologous marrow cells, a putative therapeutic measure for heritable disorders. Competitive repopulation defines PHSC populations *in vivo*. Cells to be tested are mixed with genetically distinguishable marrow. These "compete" to repopulate stem cell depleted recipients. Using correlations, and the binomial formula with covariance, most or all cells of the immune and myeloid systems are descended from very rare precursors - only about 1.0 per 100,000 transplanted marrow cells - after 3 to 12 months. The same precursors function continuously, producing differentiated cells over many months.

Stem cells that differentiate early (3-6 weeks) into both lymphoid and myeloid lines, fail to repopulate over the long term (12 or more weeks). This is surprising, as maximal differentiating and repopulating abilities are expected in the same cell type. It requires a change in stem cell definitions, since primitive hemopoietic stem cells have traditionally been defined by both abilities. Before transplantation, can long term functional multilineage precursors (PHSC) be distinguished from those that only function for a few weeks? They appear to be different populations, because 14 day fetal liver and adult marrow repopulate about equally after 3 - 4 weeks, but fetal cells are several times better after 4 - 6 months. The most simple explanation is that fetal liver contains more long term stem cells, relative to short term, than does adult marrow. Thus they are not the same cell. Myeloid specific precursors form colonies, but are not important in repopulation. From 3-50 weeks after transplantation, no better correlations occur among myeloid cells than between myeloid and lymphoid cells.

Other functional measures of PHSC include: (1. Enriched hemopoietic stem cells repopulated 1,400 - 1,600 times that as well as fresh marrow, the highest long-term repopulating ability yet measured. (2. Splenic PHSC activity increased 5-10 fold after 7 daily injections of Sl factor; this required rapid PHSC cycling, as it was vulnerable to 5FU on day 4 of the Sl factor treatment. (3. Umbilical-cord blood is a clinically useful source of stem cells, but long term repopulating ability cannot be measured in human cells. In the mouse model of 18 day fetal or newborn blood, long term repopulating abilities for equal numbers of nucleated cells are 1/3 of fresh marrow. Thus functional PHSC appear to be present in newborn and cord blood. However numbers of nucleated cells may need to be several times higher than when using marrow.

Human Hematopoietic Stem Cells

H 002 RESTRICTIONS IN THE PROLIFERATIVE POTENTIAL OF HEMATOPOIETIC STEM CELLS, Peter M. Lansdorp[1,2], Wieslawa Dragowska[1], Homayoun Vaziri[3], Calvin B. Harley[3], Vivienne Rebel[1], Connie J. Eaves[1,2], R. Keith Humphries[1,2], and Hector Mayani[1], [1]Terry Fox Laboratory, B.C. Cancer Agency, [2]University of British Columbia, Vancouver, B.C., Canada and [3]Geron Corporation, Menlo Park, CA.

The mechanism by which hematopoiesis is maintained throughout the life-span of an individual is incompletely understood. Two competing models postulate that hematopoiesis is maintained by 1) self-renewal divisions or 2) clonal succession of a population of hematopoietic stem cells. The distinction between these two models is important for a variety of experimental therapeutics strategies involving e.g. gene transfer and transplantation. In order to address this issue, we have studied the behaviour of highly purified stem cell "candidates" cultured in different well-defined culture conditions. The results of these studies can be summarized as follows. Primitive $CD34^+CD45RA^{lo}CD71^{lo}$ hematopoietic cells purified from adult bone marrow, umbilical cord blood and fetal liver show striking differences in their ability to produce $CD34^+$ cells in cytokine supplemented serum-free cultures. Both the fraction of responding cells and their proliferative potential decrease markedly with the age of the cell donor. These results suggest that the actual *production* of very primitive hematopoietic cells may be restricted to early stages of development. Selection of Thy-1^+ among $CD34^+CD45RA^{lo}CD71^{lo}$ bone marrow cells did not alter the major conclusion of the previous studies: no net production of primitive hematopoietic cells in culture. Similar findings have been obtained in a murine model in which $Sca^+Lin^-WGA^+$ adult bone marrow cells gave rise to large numbers of daughter cells including CFU-S and cells with a $Sca^+Lin^-WGA^+$ phenotype without a net increase (or decrease) in the number of cells with the ability to reconstitute long-term lympho-myelopoiesis *in vivo*. In agreement with the notion that stem cells in the adult may have limited or no "self-renewal" potential are recent observations obtained from telomere analysis. Loss of telomeric DNA at chromosome ends upon cell division has been linked to cellular senescence and aging and is thought to normally limit the proliferative potential of somatic cells. Initial studies have revealed a striking decrease in the average telomere length of hematopoietic cells from adult bone marrow compared to those from fetal liver indicating that telomere loss occurs during hematopoiesis *in vivo*. We furthermore found that cell divisions of purified $CD34^+CD45RA^{lo}CD71^{lo}$ cells *in vitro* is associated with a predictable decrease in the mean telomere length in their progeny. Taken together, these observations suggest that the proliferative potential of hematopoietic (stem) cells is not unlimited and that loss of telomeric DNA during successive cell divisions may contribute to the loss of this potential. From a practical point of view, these observations favour the use of relatively large numbers of adult hematopoietic cells for transplantation or the use of fetal or cord blood cells assuming that ontogeny-related differences in the function of cells are restricted to hematopoietic cells and do not also apply to the cells of the microenvironment.

H 003 Primitive Hematopoietic and Mesenchymal Stem Cells. Shiang Huang, Mai Nguyen, Johanna Olweus, Edmund Waller, Fridtjof Lund-Johansen, Leon.W.M.M. Terstappen. Becton Dickinson Immunocytometry Systems, San Jose, CA

Hematopoietic and stromal bone marrow progenitors express the CD34 antigen, the majority of the CD34+ cells are however lineage committed. The expression of the CD38 antigen on CD34+ bone marrow cells indicates commitment into the hematopopietic cell lineages. Fetal bone marrow CD34+, CD38- cells comprise approximately 0.6% of low density fetal bone marrow suspensions and can be separated in two cell populations based on the expression of HLA-DR. Whereas the CD34+, CD38-, HLA-DR+ population was highly enriched for cells which could give rise to each of the hematopoietic cell lineages only few of the CD34+,CD38-, HLA-DR- cells showed hematopoietic growth potential. The CD34+,CD38-,HLA-DR- cells however contained stromal cells and a few common stem cells (CSC) which sequentially can give rise to the bone marrow microenvironment and hematopoietic progeny when sorted in culture medium supplemented with serum, b-FGF and IGF-1. During the first days of culture the progeny of the single sorted CSC grow as adherent cells which gradually give rise to complex structures followed by the appearance of hematopoietic colonies on the border of these structures after 20 days of culture. The wells containing the complex structures were disaggregated after three weeks of culture and the cells were dispersed into 10 wells containing the culture medium supplemented with serum, b-FGF and IGF-1, the structures reappeared in a similar timely fashion and contained hematopoietic as well as stromal cells 3 weeks after replating. This process was repeated at three week intervals. From the 2nd generation to the 6th generation almost all of the wells contained complex structures derived from single common stem cells dramatically expanding the number of structures. Cell colonies were attached to each of the structures and when picked and plated into medium supplemented with IL-3, IL-6, Epo, GM-CSF and SCF gave rise to each of the hematopoietic cell lineages. After the 6th generation the efficiency of the structure formation gradually decreased: 79% (7th), 64%(8th), 56%(9th), 42%(10th), 19%(11th), 6%(12th) of the wells formed structures and no structures were found after the 13th replating (39 weeks after sorting a single cell). In many of the wells which did not form structures only adherent cells were found. These results suggests that the common stem cells can be expanded in vitro and can serve as a supply of hematopoietic progenitors.

H 004 CHARACTERIZATION OF THE PHENOTYPE OF VARIOUS SOURCES OF HUMAN HEMATOPOIETIC STEM CELLS.
A. Tsukamoto, L. Murray, J. Altenhofen, V. Bazil, B. Chen, S. Chen, J. Combs, A. Conti, D. DiGiusto, R. DiGiusto, S. Jagannath*, D Mandich, E. Press, S. Tindle*, G. Tricot*, N. Uchida, SyStemix, Inc., Palo Alto, CA; *University of Arkansas for Medical Sciences, Little Rock, AK.

We have previously reported the phenotype of human fetal bone marrow stem cells to be $CD34^+Thy1^+Lineage^-$. The multilineage potential of these cells was demonstrated using a long-term in vitro SyS1 coculture assay and in vivo SCID-hu mouse assays. Using these assays, we have found that adult bone marrow and peripheral blood mobilized with particular therapeutic strategies also contain $CD34^+Thy1^+Lin^-$ cells. Adult bone marrow $Thy1^+$ cells consistently gave a higher frequency of growth of clusters of refractile cells in the long-term SyS1 coculture assay and produced both B and myeloid cells compared to the $Thy1^-$ cells. Moreover, these $Thy1^+$ cells could engraft and give rise to T, B, and myeloid cells in the SCID-hu assays suggesting these $CD34^+Thy1^+Lin^-$ are stem cells. The $CD34^+Thy1^+$ cells in adult bone marrow were also shown to be largely $CD38^-$ and $HLA\text{-}DR^{lo/-}$. Moreover, of the $rhodamine^{dull}$ population about 40% are $CD34^+Thy1^+$. An analysis of the phenotype of stem cells in mobilized peripheral blood (MPB) was also undertaken. Our most extensive study examined the stem cell content of serial MPB samples from multiple myeloma patients receiving cyclophosphamide + GM-CSF. Levels of $CD34^+$, $CD34^+Lin^-$, and $CD34^+Thy1^+Lin^-$ cells in these sequential samples were recorded and found to vary greatly between patients. Moreover, the criteria for apheresis collection (WBC >500; Plts >50,000) did not necessarily correspond to the period when peak values of $CD34^+$ or $CD34^+Thy1^+Lin^-$ cells could be obtained. Significantly, the levels of $CD34^+Thy1^+Lin^-$ cells dropped precipitously over the apheresis collection period. Examination of the MPB $CD34^+Thy1^+$ cells for the expression of CD38 and HLA-DR showed that marker expression on this population may be more heterogeneous than seen for bone marrow cells. These findings suggest that there may be more heterogeneity of MPB cells; the significance of these findings will be discussed.

Stem Cells in the Nervous System I

H 005 SIGNALS THAT CONTROL CELL IDENTITY AND PATTERN IN THE NEURAL TUBE, Toshiya Yamada, Samuel L. Pfaff, Konrad Basler, *Thomas Edlund and Thomas M. Jessell, Center for Neurobiology and Behavior, Howard Hughes Medical Institute, Columbia University, New York and *Department of Microbiology, University of Umeå, Sweden.

The early organization of the vertebrate nervous system is established by the differentiation of distinct cell types at specific positions within the neural tube. In the caudal region of neural tube that gives rise to the hindbrain and spinal cord, floor plate cells and motoneurons differentiate ventrally and neural crest cells derive from the dorsal neural tube. The events that specify the identity of the distinct cell types found along the dorso-ventral (D-V) axis in the neural tube are, however, not well understood. Cell lineage studies in developing neural tube in chick and frog have shown that different neural cell types are generated from multipotential progenitor cells. We have performed in vivo grafting experiments in chick embryos and used cell type-specific markers to examine the signals that regulate the pattern of cell differentiation. Floor plate cells, motoneurons and other ventral cell types are induced by signals from the notochord and floor plate. The overall pattern of cell differentiation along the D-V axis is also altered by these manipulations, suggesting that the inductive signals from these ventral midline cells have a critical role in cell patterning in the neural tube.

To characterize further the nature of these inductive signals, we have analyzed the differentiation of cell types in neural plate explants. Motoneurons and neural crest cells differentiate in vitro from appropriate regions of the neural plate, indicating that the specification of cell fate along the D-V axis begins at neural plate stage. Motoneurons can be induced by a diffusible factor that derives initially from the notochord and later from the floor plate. In contrast, floor plate differentiation requires a contact-mediated signal from the notochord. Thus the determination of ventral cell types appears to involve distinct diffusible and contact-mediated signals from the notochord and floor plate.

Dorsal cell types differentiate independently of signals from the ventral neural tube, suggesting the existence of other factors involved in cell patterning along the D-V axis. We have cloned and characterized one candidate molecule termed *dorsalin-1* (dsl-1), a novel member of TGF-β gene family. Dsl-1 expression in the neural tube is restricted in the dorsal part and is suppressed by the notochord-derived signals. Dsl-1 protein promotes neural crest-like cell differentiation and suppresses motoneuron differentiation in vitro. These observations suggest that ventrally-derived signals and dorsally derived secreted proteins such as dsl-1 control cell fate along the D-V axis of the neural tube.

Stem Cells in the Nervous System II

H 006 GENERATION, DIFFERENTIATION, AND MATURATION OF OLFACTORY RECEPTOR NEURONS *IN VITRO*. Anne L. Calof, Murali D. Adusumalli, Melinda K. DeHamer, Jose L. Guevara, Jeff S. Mumm, and Sara J. Whitehead. Department of Biological Sciences, University of Iowa, Iowa City, Iowa 52242.

Precise regulation of neuron number during development appears to be achieved by a balance of neuron production and neuron survival: an excess of neurons is produced during the phase of neurogenesis, and then those neurons that do not receive trophic support are eliminated by a process of apoptosis, or programmed cell death (PCD). We are studying these processes in a model germinative neuroepithelium, the olfactory epithelium (OE) of the mouse embryo. Analysis of ^{3}H-thymidine (TdR) incorporation by cells in OE cultures indicates that the final step in the genesis of olfactory receptor neurons (ORNs) involves division of a migratory cell that appears to be a committed neuronal progenitor cell. We have called this cell the INP, for "Immediate Neuronal Precursor" of ORNs. In OE cultures infected with a *lacZ*-encoding murine retrovirus (in order to visualize the progeny of dividing cells), pairs of neurons arise from infected cells, consistent with the idea that the ORN precursor (the INP) is committed to a neuronal fate. We have tested several families of polypeptide growth factors for mitogenic effects on INPs. Treatment of OE cultures with Fibroblast Growth Factors (FGFs) causes a significant increase in the number of migratory cells incorporating ^{3}H-TdR from 24-48 hours in culture, a period when neurogenesis has virtually ceased in the absence of growth factor stimulation. Members of the Platelet Derived Growth Factor, Neurotrophin, Epidermal Growth Factor, and Transforming Growth Factor-β families, however, do not have this effect. Pulse-chase experiments show that ≥80% of migratory cells incorporating ^{3}H-TdR in FGF2-treated cultures also acquire immunoreactivity for NCAM (a neuronal marker in this system) within 24 hours of an early pulse, indicating that the dividing cell population is composed primarily of INPs. 3-4% of migratory cells in early (8-10 hrs *in vitro*) cultures are reactive with an antibody to the transcription factor MASH1, but <5% of cells labeled by a 10-hour pulse of ^{3}H-TdR are $MASH1^+$, suggesting that MASH1-expressing cells may be a distinct subpopulation of OE progenitor cells. To determine the mechanisms that regulate survival and maturation of ORNs, we study these processes in ORNs that develop in culture from dissociated neuronal cells purified from late embryonic OE. Assessment of ORN maturation is simplified by using cells from OT-2 transgenic mice, in which a reporter gene (Thy1.1) is expressed under the control of regulatory elements of the Olfactory Marker Protein (OMP) gene. OMP is expressed *in vivo* by mature ORNs that have made synaptic contacts with their target tissue, the olfactory bulb of the brain, and transgene expression in OT-2 homozygotes appears to replicate faithfully that of endogenous OMP . In defined, serum-free culture, dissociated OE neuronal cells attach and initially extend neurites, but die within 72 hours. In the presence of aurintricarboxylic acid (AT), an agent that inhibits PCD in other cells, a significant fraction (>15%) of neuronal cells survive for 72 hours. Interestingly, in these cultures the number of Thy1.1-expressing ORNs surviving for 72 hrs appears to be greater than the number of Thy1.1-positive cells that were present at the time of initial plating. This suggests that maturation of ORNs may not require trophic support from target tissue, but may instead follow an intrinsic developmental program that is uncovered when PCD is inhibited.

Factors and Receptors for Neural Cells

H 007 THE ROLE OF NEUROPOIETIC CYTOKINES IN NEURAL DEVELOPMENT AND INJURY, Paul H. Patterson, Biology Division, California Institute of Technology, Pasadena, CA 91125.

As in the hematopoietic system, the enormous variety of phenotypes in the nervous system arises, in part, through the action of instructive differentiation signals. Neuronal culture assays have allowed the identification and cloning of several proteins that control the expression of phenotype-specific genes in developing neurons. A group of cytokines induces and suppresses the expression the same set of genes coding for particular neurotransmitter synthetic enzymes and neuropeptides in cultured sympathetic neurons. Several members of this group, which now includes CDF/LIF, CNTF, OSM, and GPA, share a predicted secondary structure, as well as the binding of a transducing receptor subunit in common with IL-6 and IL-11. The latter two cytokines display a weak activity in the sympathetic neuron assay. In addition, we find that one that one member of the TGFβ superfamily, activin A, shares a selective overlap with the neuropoietic family in the spectrum of neuropeptides that it induces in sympathetic neurons. The particular neuropeptides induced by activin A, however, demonstrate that the activity of this cytokine is distinct from that of the neuropoietic family.

To study the role of CDF/LIF *in vivo*, we are localizing its mRNA and that of its receptor. CDF/LIF mRNA levels are developmentally modulated, and substantial differences are observed between tissues. Particularly high expression for both CDF/LIF and the LIFR is found in the visual system of the adult rat. In sciatic nerve and sympathetic ganglia, the postnatal increase in receptor expression parallels expression of CDF/LIF.

Analysis of mice in which the CDF/LIF gene has been targeted by homologous recombination reveals a role for this cytokine in the injury response as well as in the normal brain. CDF/LIF mRNA levels increase dramatically after damage to normal, mature peripheral nerve and ganglia, and a series of neuropeptide mRNAs are induced. This neuropeptide induction is not nearly so pronounced in the homozygous mutant mice, demonstrating a role for CDF/LIF in this injury response. In addition, the brains of the adult mutant mice display several major changes in the visual cortex and hippocampus. These alterations include a loss of pyramidal neurons and a diminution of staining for the calcium binding protein parvalbumin. Similar changes are not observed in several other areas of cortex, which do not normally express the relatively high levels of CDF/LIF mRNA observed in the visual areas. In addition, there is a dramatic increase in tyrosine hydroxylase (TH)-like immunoreactivity in several areas of the brain. TH is one of the genes whose transcription is repressed by CDF/LIF in cultured sympathetic neurons.

P.H. Patterson, Curr. Opinion Neurbiol. 2: 94 (1992); Patterson and Nawa, Cell 72: 123 (1993); Fann and Patterson, J. Neurochem. and PNAS., in press.

H 008 NEUROTROPHIC FACTORS AND HOW THEY WORK George D. Yancopoulos,Regeneron, Inc., 777 Old Saw Mill River Road, Tarrytown, NY

Naturally occurring neuronal cell death accompanies normal embryonic development, whereas abnormal neuronal death is a key feature of a variety of neurodegenerative diseases. Neurotrophic factors were discovered for their ability to prevent neuronal cell death. We have found that two distinct classes of neurotrophic factors, those related to Nerve Growth Factor (including Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 and Neurotrophin-4/5) and those related to Ciliary Neurotrophic Factor (CNTF), utilize fundamentally different receptor systems. Each of these, however, are related to receptor systems which function outside of the nervous system.

The Nerve Growth Factor (NGF) family of factors utilize receptor tyrosine kinases (the Trks) very similar to those used by traditional growth factors such as Fibroblast Growth Factor (FGF) or Platelet-Derived Growth Factor (PDGF). The Trks do, however, display several unprecedented functional features, including interactions with an additional receptor component, known as the low-affinity NGF receptor. CNTF, on the other hand, employs a three-component receptor system, of which two components (gp130 and LIFRβ, both referred to as "β" signal transducing receptor components) are also utilized by the receptor systems of certain hemopoietic cytokines that are distantly related to CNTF (such as interleukin-6 and leukemia inhibitory factor). CNTF and its cytokine relatives all initiate signaling by inducing either homo- or hetero-dimerization of their "β" signal transducing receptor components, which in turn activates cytoplasmic tyrosine kinases that are associated with these "β" receptor components. The CNTF receptor system is distinguished from those of its cytokine relatives in that it requires a third receptor component (an "α" component) that is mostly restricted to the nervous system in its expression, limiting CNTF to its actions on neural cells. Not only do the CNTF and the NGF families of neurotrophic factors utilize very different receptor systems and signaling pathways to keep a neuron alive, but these distinct signaling pathways can collaborate in remarkable ways. For example, these pathways can work together to drive the terminal differentiation of primitive, proliferating neural progenitors to produce a mature, post-mitotic neuron.

Stem Cells in Other Organs I

H 009 STEPS ON THE PATH TO PRECURSOR CELL TRANSPLANTATION FOR TISSUE REPAIR, Mark Noble, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, U.K.

Our studies on the biology of glial precursor cells of the central nervous system (CNS) will be discussed as a paradigm for the demonstrating some of the overlaps between the fields of developmental biology, regeneration research and oncology. In addition, I will discuss new tools which may greatly facilitate the study of precursors cell function in other tissues.

Our studies on the CNS have been focused largely on oligodendrocyte-type-2 astrocyte (O-2A) progenitor cell of the rat optic nerve. In vitro, this cell can be induced to give rise to oligodendrocytes or type-2 astrocytes. Astrocytes of a separate glial lineage modulate division and differentiation of O-2A progenitor cells through secretion of platelet-derived growth factor (PDGF).

Growth of O-2A progenitors in the presence of both PDGF and basic fibroblast growth factor (bFGF) induces continuous self-renewal in the absence of differentiation. Thus, cooperation between growth factors can "conditionally immortalize" these precursor cells as effectively as expression of activated oncogenes. The ability to grow enriched populations of O-2A progenitors in the absence of differentiation has allowed us to repair demyelinating lesions transplantation of purified and expanded O-2A progenitor populations .

Optic nerves of adult rats contain a different cell type, the O-2A^{adult}progenitor, with properties specialized for the physiological requirements of the adult nervous system. In particular O-2A^{adult}progenitors have many of the features of stem cells. O-2A^{adult}progenitors are derived from a subset of O-2A^{perinatal} progenitors, thus indicating that the O-2A^{perinatal} progenitor population is tripotential, rather than bipotential. These observations offer novel insights into the possible origin of self-renewing stem cells and also into the role that generation of stem cells may play in helping to terminate the explosive growth of embryogenesis. Exposure of these cells to a combination of PDGF and bFGF causes cells to go through a transient period of accelerated division, as might be required during repair of demyelinating lesions in vivo. The properties of O-2A^{adult} progenitor cells are strikingly consistent with the failure of successful myelin repair in multiple sclerosis.

In addition, we have developed transgenic mice that harbour a temperature-sensitive version of the SV40 large T antigen under the transcriptional control of the H2-K^b promotor. Growth of cells derived from these animals in tissue culture at 33^{o}C in the presence of interferon-gamma produces conditional immortalization of a variety of cell types. These animals present a new tool which may obviate the need for retroviral- or transfection mediated gene insertion in the creation of novel cell lines.

H 010 HUMAN EPIDERMAL STEM CELLS, Fiona M. Watt and Philip H. Jones, Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London. WC2A 3PX, U.K.

The epidermis is believed to contain two types of proliferating keratinocyte: stem cells and cells with a lower capacity for self-renewal and higher probability of terminal differentiation (transit amplifying cells). Keratinocytes with characteristics of stem cells can be isolated from cultured human epidermis on the basis of high surface expression of β_1 integrins and rapid adhesion to fibronectin, type IV collagen or keratinocyte extracellular matrix (1). In contrast, there is no correlation between high proliferative capacity and high surface expression of the α_6 integrin subunit or rapid adhesion to laminin. Keratinocytes with characteristics of transit amplifying cells adhere slowly to matrix proteins and express low levels of β_1 integrins: after a few rounds of division all the daughters of these cells undergo terminal differentiation. One stimulus for terminal differentiation is a reduction in the proportion of β_1 integrins on the cell surface with bound ligand (2) and the putative stem cell population can differentiate when deprived of contact with extracellular matrix proteins. However, we would propose that in vivo the high number of functional β_1 integrins on the surface of stem cells will ensure that they are less likely than transit amplifying cells to reach the threshold of receptor occupancy that triggers terminal differentiation. The variation in β_1 integrin levels that we have found in cultured keratinocytes is also observed in intact epidermis and we are currently investigating whether there is a similar relationship between expression and proliferative potential.

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2. Watt, F.M., Kubler, M-D., Hotchin, N.A., Nicholson, L.J. and Adams, J.C. (1993) J. Cell Sci. in press

Stem Cells in Other Organs II

H 011 THE PROGENITORS OF HAIR CELLS REGENERATED IN THE LATERAL LINE AND THE EAR, Jeffrey T. Corwin, Jay E. Jones, Bradley J. Goldstein, Akiko Katayama, Matthew W. Kelley, Paul R. Lambert, and Mark E. Warchol, Departments of Otolaryngology and Neuroscience, University of Virginia, Charlottesville, VA 22908

Tens of millions of individuals are affected by deafness and balance disorders that stem from loss of the sensory hair cells which transduce acoustic and accelerational stimuli into transmembrane electrical potentials in the inner ear. The clinical permanence of those disorders and the results of early studies of cell production in mammalian embryos led to the belief that hair cells could not be produced in the ear after birth. That is not the case for fish and amphibians where early investigations showed that thousands of hair cells are produced and added to the organs of the ear during postembryonic life, suggesting that lost hair cells might be regenerated. Over the past decade, regeneration of hair cells has been demonstrated in the ears and the lateral line hair cell epithelia of aquatic vertebrates, the hearing and balance organs of birds, and most recently in the balance organs of adult mammals. Evidence from a range of organs and methods, including direct visualization of cell lineage history via time-lapse microscopy in the lateral line, has indicated that the replacement hair cells originate as the progeny of supporting cell divisions. Those progeny are bipotential in that they can differentiate as supporting cells or as hair cells, when they develop at sites where hair cells are missing. In the case of the avian hearing organ and the mammalian balance organs the supporting cells that give rise to replacement hair cells during postembryonic life appear to change from arrest in a quiescent state to a proliferative state in response to trauma to the sensory epithelium. Under normal conditions the populations of supporting cells in those organs appear to be proliferatively quiescent, as shown by the absence of ^{3}H-thymidine labeling in animals that have received long-term continuous administration of the tracer. The potential for hair cell regeneration in the cochlea, which is responsible for hearing in mammals, has been examined in cultures where it was determined that hair cells killed before birth could be replaced as preexisting uncommitted cells moved into the site of a hair cell lesion and differentiated as hair cells, but this did not occur in older cultures. Hypothetical lateral inhibitory interactions between the progeny of supporting cells have been proposed, and appear to explain how the patterns of hair cells and supporting cells develop during embryogenesis and are reconstituted during regeneration. Other cues may influence an earlier commitment of cells to a "prosensory" phenotype as suggested by the demonstration that embryonic cochleae which have been treated with low concentrations of retinoic acid develop supernumerary patches containing up to thirteen rows of hair cells rather than the four to five rows that develop in vivo and in matched control cultures. A model for the production and progressive commitments of these cells has developed from this work, but questions pertaining to the factors that may trigger the proliferative response of supporting cells to trauma remain intriguing and fertile for discussion.

Transplantation of Stem Cells and Barriers to Their Transplantation

H 012 MYOBLAST MEDIATED GENE THERAPY: A NOVEL FORM OF DRUG DELIVERY, Helen M. Blau, Thomas A. Rando, and Grace K. Pavlath, Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5332.

Myoblasts are particularly advantageous for cell mediated gene transfer because following injection they cross the basal lamina, randomly fuse with all myofibers in their vicinity and become integrated into a pre-existing structure, the multinucleated myofiber[1,2]. As a result, they are in contact with the circulation and sustained by neuronal activity. In a recent clinical trial, biopsies of Duchenne Muscular Dystrophy patients 1 and 6 months after implantation with primary human myoblasts produced the missing gene product, dystrophin detected by PCR, albeit at low efficiency[3]. Myoblasts of an established cell line genetically engineered with retroviruses delivered recombinant human growth hormone to the circulation for at least 3 months[4,5] Recent studies examined whether efficient myoblast transfer could be achieved with primary myoblasts from mouse and man. Once the appropriate medium was identified, mouse myoblasts could be easily isolated, purified and expanded. Satellite cells isolated from neonatal mice were found to undergo more than 50 population doublings, yielding more than 10^{15} cells per cloned myoblast. Recombinant genes introduced by retroviral mediated gene transfer were stably expressed following transplantation of primary mouse myoblasts into the muscles of syngeneic mice. These developments should allow use of primary myoblasts of any strain in a wide range of mouse models of human disease. Human primary myoblasts from young (7 yr) and old (42 yr) donors were purified[6], genetically engineered by retroviral infection, grown for 25 to 35 doublings, and shown to stably express a recombinant gene for many weeks following transplantation into immunodeficient SCID mice. Thus, myogenic "stem cells" may have several desirable features: they are readily separated from other cells, they have extensive proliferative capacity required for genetic engineering and selection in vitro, and even when derived from "aged" donors they retain their ability to become incorporated into mature muscle fibers upon injection into host muscle tissue. Given these properties, muscle stem cells are attractive cellular vehicles for treating inherited and acquired myopathies and nonmuscle disorders including hemophilia, cancer and heart disease.

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H 013 FUNCTIONAL EFFECTS OF GRAFTING GENETICALLY MODIFIED CELLS TO THE CENTRAL NERVOUS SYSTEM.

Fred H. Gage, Department of Neuroscience, University of California, San Diego, San Diego, CA 92093-0627.

Intracerebral grafting of genetically modified cells can be used both to explore basic biological questions concerning the structure and function of the brain, or as a form of somatic gene therapy. In this technique cells are taken from the body, grown *in vitro*, transfected with new genes that make molecules of functional importance, and implanted into the brain. Recently, cells genetically engineered to secrete NGF and implanted into the brain were shown to prevent the degeneration of cholinergic neurons which normally occurs following axotomy. These NGF-producing cellular implants have been shown to not only protect cholinergic neurons from dying, but also to induce regeneration of the transected axons. More recently, injections of cells genetically modified to secrete ciliary neurotrophic factor (CNTF) brain derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) have been used to explore the selectivity and specificity of neuronal cell survival, axonal elongation, and recovery of physiological function following brain damage and aging. These recent studies in rodents and primates will be summarized and discussed.

H 014 THE ROLE OF THE LY-49 FAMILY OF RECEPTORS IN MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I SPECIFICITY OF NATURAL KILLER (NK) CELLS. Wayne M. Yokoyama[1], Brian F. Daniels[2], Hamish R.C. Smith[1], William E. Seaman[2], David H. Margulies[3], and Franz M. Karlhofer[1], [1]Molecular Medicine Division, Department of Medicine, Mount Sinai Medical Center, New York, NY 10029, [2]Department of Medicine, VA Medical Center, San Francisco, CA 94121, and [3]Lab. Immunology, NIAID, Bethesda, MD 20892.

NK cells constitute the third major population of lymphocytes. In addition to their well-known ability to kill tumor cells, NK cells possess an innate capacity to kill cells infected with intracellular pathogens and respond to cytokines and produce other cytokines. Moreover, NK cells in F_1 hybrid mice reject parental bone marrow grafts that may involve MHC class I molecules on donor cells. Recent studies also indicate that target cell surface expression of MHC class I molecules inversely correlates with susceptibility to NK cell mediated lysis but the mechanism for this is poorly understood. One hypothesis suggests that NK cells may express a receptor that may deliver "positive" signals to activate NK cells. Another receptor may specifically engage target cell MHC class I molecules and deliver "negative" signals, inhibiting cytotoxicity. Although the putative "positive" NK cell receptor remains elusive, our studies suggest that the murine Ly-49 molecule may be an NK cell inhibitory receptor that is specific for MHC class I molecules.

Ly-49 is a type II integral membrane protein with an extracellular carboxy terminal domain homologous to the Ca^{++}-dependent lectins. It is expressed by a distinct NK cell subpopulation. Ly-49$^+$ and Ly-49$^-$ subsets of IL-2-activated NK cells have comparable abilities to spontaneously kill certain murine targets. However, target cells of the H-2^d and H-2^k haplotype were resistant to killing by Ly-49$^+$ NK cells. When a susceptible tumor cell line was transfected with cDNAs encoding various MHC class I molecules, only cells transfected with D^d became preferentially resistant to spontaneous killing by Ly-49$^+$ NK cells. Gene-transferred resistance globally inhibited activation of Ly-49$^+$ NK cells through physically distinct pathways and was overcome by appropriate mAbs. This suggested that Ly-49 may engage D^d molecules on target cells. To more directly demonstrate this physical interaction, we expressed Ly-49 at high levels on dhfr-deficient CHO cells by methotrexate-amplification. In rosette formation and quantitative cell-cell binding assays, we demonstrated that cells expressing transfected D^d, but not L^d or K^d, or parental cells, bound specifically to the Ly-49$^+$ CHO cells. The D^d-transfectant did not bind to CHO cells containing an anti-sense Ly-49-dhfr construct. Specificity was also confirmed by mAb blocking studies. MAbs specific for Ly-49 or $\alpha1/\alpha2$ domains of D^d inhibited binding but control mAbs and a mAb specific for the $\alpha3$ domain of D^d had no effect. Thus, these studies suggest that Ly-49 engages target cell MHC class I molecules and delivers inhibitory signals.

If the interaction between Ly-49 and target cell MHC class I molecules represents a paradigm for NK cell specificity, we reasoned that Ly-49$^-$ NK cells should express other molecules that may be structurally related to Ly-49 and provide a similar function to these cells. We have recently isolated cDNAs by cross-hybridization and PCR that encode molecules that are significantly homologous to Ly-49. These molecules are expressed by NK cells that do not express Ly-49. Thus, the Ly-49 family of molecules may determine NK cell specificity for MHC class I molecules expressed by their targets.

Presenters A-H

H 100 GENERATION OF NATURAL KILLER CELLS FROM PURIFIED THY1.1^{lo}LIN$^{-/lo}$SCA^{+} BONE MARROW CELLS IN VIVO, Hector L. Aguila, Nobuko Uchida and Irving L. Weissman. Department of Pathology and Developmental Biology. Stanford Medical Center. Stanford, CA 94305.

Natural killer (NK) cells have been well defined in basis of their ability to kill defined cell targets. It is known that NK cells can be generated from bone marrow precursors, but their relationship with the different hematopoietic lineages and their development to mature functional cells is not clear.

We tested whether NK cells are derived directly from hematopoietic stem cells (HSC). We have analyzed the generation of NK cells, defined by the NK1.1 marker, from purified Thy1.1^{lo}Lin$^{-/lo}$Sca^{+} bone marrow cells. These cells, a candidate population for mouse HSC, can reconstitute the whole NK cell compartment. Donor derived NK cells appear, in spleen and in bone marrow, as early as 14 days after reconstitution and their appearance is independent of the presence of thymus. Competitive repopulation studies between HSC and Sca^{-} cell fractions from bone marrow evidence no contribution of the Sca^{-} population to the donor derived NK cell compartment, even when these cells represent 95 % of the bone marrow cells..

With the purpose of define lineage relationships and/or differentiation pathways, the coexpression of lymphoid (Thy1.1 and B220) and myeloid (Mac 1) markers was also investigated . Differential expression of these markers was observed in bone marrow vs. spleen. For example, a major representation of Thy1.1+ cells is found in NK1.1+ cells from bone marrow, while the reverse was found for Mac 1 expression. These differences are evident at every time after the appearance of donor derived NK cells. The coexpression of markers after reconstitution is discussed in basis to the influence of microenvironment in the differentiation of NK cells and NK cell progenitors from hematopoietic stem cells in mouse.

H 101 **MOLECULAR CLONING OF A LIGAND FOR THE FLT3/FLK-2 RECEPTOR - A PROLIFERATIVE FACTOR FOR EARLY HEMATOPOIETIC CELLS.** M. Patricia Beckmann, Tim Vanden Bos, Laura James, Ken Brasel, Peter de Vries, Kathleen S. Picha, Terry Farrah, L. T. Hollingsworth, Brian Gliniak, Hilary J. McKenna, Frederick A. Fletcher, Roxanne R. Splett, Eugene Maraskovsky, Douglas E. Williams, and Stewart D. Lyman. Immunex Research and Development Corp., Seattle, WA 98101.

Characterization of a ligand for the flt3/flk-2 receptor was undertaken using a soluble form of the receptor to identify a source of the ligand. A murine T cell line was identified that expressed the ligand on its cell surface. Screening of a cDNA library prepared from these cells using a two-step direct-expression strategy allowed isolation of a clone encoding a transmembrane protein capable of binding to the flt3/flk-2 receptor molecule. This protein has similarities in both size and structure to Steel factor and colony stimulating factor 1. Cross hybridization of the murine ligand to a human T cell cDNA library allowed isolation of a human flt3 ligand cDNA which is 72% identical to the murine ligand. The flt3/flk-2 receptor has been reported to be expressed on murine fetal liver stem cells. We tested a soluble form of the flt3 ligand for its biological effects on c-*kit* positive adult murine bone marrow stem cells and AA4.1 positive murine fetal liver cells. The flt3 ligand stimulates the proliferation of each of these cell types, and synergizes with some hematopoietic growth factors. The murine factor also stimulates the proliferation of human CD34 positive bone marrow cells.

H 102 EXPRESSION OF LIVER TRANSCRIPTION FACTORS AFTER ACTIVATION OF THE PUTATIVE HEPATIC STEM CELL COMPARTMENT IN THE RAT. Hanne Cathrine Bisgaard, Peter Nagy, and Snorri S. Thorgeirsson. Laboratory of Experimental Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The involvement of a hepatic stem cell compartment in the restoration of the liver mass after experimental injury in the rat is now well documented in several models. In an attempt to better understand the role of the different liver transcription factors in the regulation of hepatic stem cell differentiation, we have examined the cellular expression of the four major families of "liver specific" transcription factors during the differentiation process. The temporal and spatial distribution of their transcripts as assessed by in situ hybridization analysis indicates that the factors are regulated individually and may play crucial roles at the different stages of stem cell activation and differentiation in the adult liver. Most of the "establishment transcriptional factors" including HNF1α and HNF3γ were upregulated in small proliferating epithelial cells thought to be a progeny of putative stem cells when compared to surrounding hepatocytes. Interestingly, upregulation of HNF4 was first observed in newly formed hepatocytes, suggesting that this factor plays a key role in the final hepatocyte lineage commitment of the putative stem cells.

H 103 NBGF, A GROWTH FACTOR ACTIVITY SECRETED BY A WILMS' TUMOR CELL LINE (G401), ENABLES THE CONTINUOUS CULTURE OF HUMAN RENAL PROGENITOR CELLS, Christopher R. Burrow and Patricia D. Wilson, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Mammalian renal development is an example of embryonic induction of mesoderm to epithelium. Undifferentiated metanephric blastemal cells (nephroblasts) are induced by the branching ureteric bud to undergo a series of molecular and morphological events leading to their conversion to the several epithelial cell types characteristic of the nephron. A novel in vitro system has been developed in which human nephroblast progenitor cells can be grown indefinitely in cell culture and that these cells retain their capacity to differentiate in response to serum and/or a type !V collagen extracellular matrix. This has been demonstrated by morphological and immunocytochemical marker analysis. Continuous culture of undifferentiated renal progenitors (vimentin, syndecan, N-CAM positive, cytokeratin, E-cadherin, PAX-2, alkaline phosphatase-negative) was only possible in the presence of serum-free, conditioned medium collected from G401cells derived from a Wilms' tumor containing a soluble growth factor activity (NB-GF). To address the physiological relevance of epithelial conversion in vitro by removal of NB-GF and addition of 10% serum to the ureteric induction process in vivo, PAX-2 regulation was studied (antibody generously supplied by G. Dressler). Immunostaining of human fetal kidney tissue sections show that induction of PAX-2 was an early event at the onset of induction and that its expression was transient. This temporal pattern of expression of PAX-2 was closely mimicked in vitro since in the absence of serum and presence of NB-GF, nephroblasts were PAX-2 negative while shortly after serum stimulation PAX-2 was clearly expressed in cell nuclei. After longer exposure to serum, however, fully converted, cytokeratin-positive epithelia no longer showed PAX-2 staining. Our results suggest that this cultured cell system represents early, uninduced renal progenitors and will be suitable for the study of differentiation in general and the role of PAX-2 in mesodermal induction in particular.

H 104 EVIDENCE THAT AcSDKP CAN BLOCK THE CYCLING OF PRIMITIVE NORMAL BUT NOT LEUKEMIC PROGENITORS BY AN INDIRECT MECHANISM INVOLVING MACROPHAGE INFLAMMATORY PROTEIN-1 ALPHA. J.D. Cashman, A.C. Eaves and C.J. Eaves. Terry Fox Laboratory, British Columbia Cancer Agency and University of British Columbia, Vancouver, B.C., V5Z 1L3, Canada.

In steady-state hemopoiesis, the majority of primitive (high proliferative potential) clonogenic progenitors are found in G_0 whereas most of their Ph-positive counterparts in CML proliferate continuously. This differential control of primitive normal and neoplastic cells is also reproduced in the adherent layer of long-term cultures (LTC) of normal and CML cells, respectively. We have previously shown that the ability of stromal cells to downregulate the proliferation of primitive normal cells in LTC involves the endogenous production of TGF-β and a factor (like MIP-1α) that is specifically antagonized by MIP-1β. The failure of CML progenitors to be downregulated in this system appears to be due to their selective insensitivity to MIP-1α (but not TGF-β). In the present series of experiments we have shown that addition of the tetrapeptide AcSDKP (but not related control peptides, AcSDKE or SDKP) at concentrations of ≥50 ng/ml to normal cultures, like the addition of MIP-1α, can block the activation of primitive but not more mature clonogenic progenitors. In contrast the addition of AcSDKP to CML cultures, like the addition of MIP-1α, was ineffective in arresting the proliferation of primitive neoplastic progenitors. Since cell separation experiments revealed that the ability of AcSDKP to inhibit colony formation by normal progenitors in methylcellulose assays might be indirect, we tested whether the effect of AcSDKP addition to normal LTC might also be indirect and possibly blocked by the simultaneous addition of MIP-1β. Results from 4 experiments revealed this to be the case (% kill values for primitive normal progenitors in control cultures given a regular medium change = 69 ± 9, with 200 ng/ml AcSDKP = 2 ± 1, with 200 ng/ml AcSDKP + 300 ng/ml MIP-1β = 50 ± 13). These experiments suggest an interesting potential for the future use of AcSDKP to stimulate the local production of a factor(s) that may differentially protect primitive normal cells from the cytotoxic activity of cell cycle-specific drugs thereby increasing the possibility of an increased therapeutic index with decreased systemic toxicity and without the need for exogenous hemopoietic cell rescue.

H 105 DERIVATION AND CHARACTERIZATION OF A NOVEL TYPE OF MOUSE EMBRYONIC STEM CELL FROM PRIMORDIAL GERM CELLS

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Primordial germ cells (PGCs) are embryonic precursor cells of the germ line in vertebrates. Mouse PGCs are first identified at day 7.0 of gestation as a small population cells with alkaline phosphatase (AP) activity. During the subsequent 5 to 6 days of development, they migrate to the gonad analgen and proliferate rapidly to establish a population of as many as 25,000 germ cells. Shortly after arrival in the gonad PGCs stop mitotic cell division, and enter mitotic arrest in the developing testis or meiosis in the ovary at day 13 of gestation. By using genetic and cell culture analysis we and others have identified three growth factors required for PGC survival and proliferation: a. Steel factor (SLF, also called Stem Cell Factor, Mast Growth Factor or KL since it is the ligand for the *c-kit* tyrosine kinase receptor); b. Leukemia Inhibitory Factor (LIF); and c. basic Fibroblast Growth Factor (bFGF). Combination of the three factors allows continuous PGC growth and the derivation of cell lines which maintain PGC markers such as AP activity and the SSEA-1 antigen.

Strikingly the cells derived from primary PGCs, termed embryonic germ cells or EG cells, resemble mouse embryonic stem (ES) cells which also express AP and the SSEA-1 antigen. We have examined the developmental potential both *in vivo* and *in vitro*. EG cells can be induced in culture to generate embryonic bodies containing various types of differentiated cells. When re-introduced into host blastocysts, EG cells can give rise to chimeric animals in the same way as ES cells. Thus mouse EG cells we established can function as embryonic stem cells with pluripotent developmental capacities.

Results of our investigation on the roles of SLF, LIF and bFGF in PGC development, and for establishing and maintaining EG cells will be discussed in details. Knowledge gained from this study on the derivation and characterization of EG cells may may be useful for establishing stem cell lines from other tissues, and for generation of embryonic stem cells from other species.

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H 106 IDENTIFICATION OF STEM AND TRANSIENTLY AMPLIFYING POPULATIONS IN HUMAN EPIDERMIS; INCREASED GROWTH RESPONSIVENESS OF PSORIATIC KERATINOCYTE STEM CELLS TO LESIONAL PSORIATIC T CELLS. K.D. Cooper, Zs. Bata-Csorgo, J.J. Voorhees, and C. Hammerberg, Dept. of Dermatology, University of Michigan, Ann Arbor, MI.

Radioactive labeling techniques do not address the heterogeneity of the proliferating populations in the epidermis. We obtained fresh epidermal cells from keratome skin biopsies and utilized flow cytometric analysis to study in vivo proliferative compartments of human epidermis. We were able to simultaneously follow four different parameters: 1) cell size and granularity; 2) expression of the predominantly basal cell α_1 integrin CD29; 3) expression of the predominantly suprabasal K1 & K10 keratins; and 4) DNA content or PCNA expression of individual cells. In normal epidermis all cells in the proliferative compartment are exclusively $CD29^+$, but this is divided into the $CD29^+$ cells two distinct populations.. The $CD29^+$ $K1/K10^-$ small cell population contains very few cells in cell cycle (2.5%), and may include slow-cycling stem cells. Large $CD29^+$ cells contain a bigger cycling pool (S/G2/M:18.6%), have begun differentiation ($K1/K10^+$), and may include the transiently amplifying cells (TAC). The $CD29^+$ $K1/K10^-$ (stem) population in psoriasis exhibits a 9 fold increase in its cycling pool over normals, whereas the $CD29^+$ $K1/K10^+$ population, albeit enlarged, contained a normal cycling pool. Thus, the $CD29^+$ $K1/K10^-$ stem cell subset appeared to represent the primary proliferating compartment responsible for hyperproliferation in psoriasis. Indeed, supernatants from growth stimulatory T cell clones derived from lesional psoriatic skin induced an increase in both the proportion of stem cells in fresh ex vivo epidermal cultures (9.65±3.9% versus 3.37±1.1% in control media, n=4) and in the % of stem cells in S/G2/M phase of the cell cycle (2.85±0.8% versus 1.2±0.27%, n=4) in psoriatic uninvolved epidermal cell cultures. By contrast, when the activated T cell supernatant was added to fresh epidermal cells obtained from normal skin, we could not observe growth induction. Conclusion: a) stem cells and TAC's can be physically identified by flow cytometry; b) the stem cell is the primary source of hyperproliferation in psoriasis; c) psoriatic T cells stimulate proliferation of basal stem cells; d) psoriatic, but not normal, stem cells grow in response to T cell stimulation.

H 107 EVIDENCE FOR A RELATIVELY QUIESCENT STEM CELL IN THE ADULT MAMMALIAN FOREBRAIN, C.G. Craig, C. Morshead, A. Roach* and D. van der Kooy, Depts. of Anatomy and Cell Biology, and *Molecular and Medical Genetics, Univ. of Toronto; and *Samuel Lunenfeld Research Inst., Mt. Sinai Hospital, Toronto, CANADA.

In adult brain, the subependymal region lining the lateral ventricles contains a unique subpopulation of cells that constitutively proliferate. The adult subependymal region has also been shown to give rise to a stem cell *in vitro* that can differentiate into both neurons and glia. Experiments utilizing retrovirus infection of these proliferating cells *in vivo* and subsequent β-galactosidase (β-gal) reporter gene expression as a non-diluting marker have found that with increasing adult mice survival times of up to 28 days post retrovirus infection there is a progressive loss of β-gal positive subependymal cells. Relative to 1 day survival animals, 6 days following retrovirus infection there is a 45% loss of β-gal positive cells and 28 days following retrovirus infection there is a 97% loss. This progressive loss of retroviral β-gal expressing cells could be due to either a decrease in expression of the retrovirally introduced gene or to the loss of the retrovirally infected cells through cell death. To differentiate between these two possibilities, histochemically β-gal positive and nearby negative regions of retrovirally infected brain slices were assayed for retroviral DNA by nested PCR to determine whether or not β-gal negative regions include cells containing non-expressed retroviral DNA. This assay proved to be specific for retroviral DNA (sensitive down to 10pg of retroviral genome containing DNA) and the majority of regions containing single histochemically β-gal positive cells also gave positive PCR signals. If the major mechanism for the temporal loss of β-gal cells is progressive turn off of retrovirally introduced genes, then a progressive increase in the non-β-gal expressing but PCR positive regions would correlate with increasing survival times post infection. Of the non-β-gal expressing regions tested, 21% of those regions from day 1 post infection brain slices resulted in a positive PCR signal (probably due to non-integrated transient retroviral DNA), whereas 6 days following infection this percentage had decreased to 1.7% and 28 days following infection there were 0% PCR positive signals in these regions. The data do not support a role for a down regulation of β-gal gene expression over time, rather the temporal loss of β-gal positive cells in retrovirally infected subependymal cells must be the result of the progressive death of these cells over time. Thus, maintainance of the constant number of proliferating subependymal cells seen throughout adult life suggests that a relatively quiescent stem cell must proliferate sporadically both to replenish the constitutively proliferating cell population and to self-renew.

H 108 CHARACTERIZATION OF THE LIGAND FOR FLT3/FLK2. Janice Culpepper, Chuck Hannum, Jeanine Mattson, Jeanne Luh, Terrill McClanahan, David Campbell, Greg Duda, Sandy Zurawski, Janet Wagner, Natalie Martina, Donna Rennick, David Peterson, Rob Kastelein, Armen Shanafelt, Satish Menon, Warren Dang, Fernando Bazan, Olivier Rosnet, Patrice Dubreuil, Daniel Birnbaum and Frank Lee. DNAX Research Institute, Palo Alto, CA 94304 and INSERM, 13009 Marseilles, France.

Flt3 (also called Flk2) is a receptor tyrosine kinase most homologous to c-kit and c-fms, the receptors for stem cell factor and M-CSF, respectively. Flt3 messenger RNA is found in populations of cells highly enriched for hematopoietic stem cells as well as in progenitor cells. It is also found in fetal liver and brain, and adult brain, thymus, and placenta. The ligand for this receptor may share similarities with SCF and M-CSF. We will report the purification, sequencing, and partial characterization of the Flt3 ligand derived from a mouse thymic stromal cell line TA4. In addition, we will present the molecular cloning of the Flt3 ligand and some of the biological properties of the expressed recombinant protein.

H 109 *TROL* AND *ANA*: REGULATION OF NEUROBLAST PROLIFERATION IN THE *DROSOPHILA* CENTRAL NERVOUS SYSTEM. Sumana Datta, Department of Biochemistry and Biophysics and Department of Biology, Texas A&M University, College Station, Texas, 77843

The *Drosophila* larval central nervous system is an excellent system to examine the regulation of neuroblast proliferation due to the stereotyped pattern of neuroblast birth and cell division. Lack of the cellular migration which typifies mammalian and vertebrate systems makes possible the reproducible identification of specific neuroblasts and their progeny. I have previously identified a mutation at the *trol* locus that causes a 90% drop in the dividing cell population of the larval central nervous system (1). Studies of *trol* mutants at third larval instar suggest that low levels of *trol* expression are sufficient for the proliferation of some lineages in the larval brain lobes, but not for most lineages in the thoracic portion of the ventral ganglion. This may account for the lethality of the mutation at early third instar. Preliminary results suggest that the proliferative defect is visible soon after the first instar larva hatches from the egg. The *anachronism* (*ana*) locus has been identified and characterized by the Zipursky laboratory (2). *ana* encodes a secreted glial glycoprotein that represses premature neuroblast proliferation in the larval brain. *ana trol* double mutants were constructed to examine developmental hierarchies in the regulation of neuroblast proliferation in the developing brain. Preliminary results indicate that double mutant individuals die at early third instar, as do *trol* mutants. In addition, the segmental abdominal neuroblasts, which are proliferating in *ana* mutant brains, are not proliferating in *ana trol* double mutant brains at mid second instar.

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H 110 THE EFFECT OF THE FLT3 LIGAND ON PURIFIED MURINE PLURIPOTENT HEMATOPOIETIC STEM CELLS. Peter de Vries, Kenneth A. Brasel, Tim Vanden Bos, Laura James, M. Patricia Beckman, Hilary J. McKenna, Brian C. Gliniak, L.T. Hollingworth, Kathleen S. Picha, Roxanne R. Splett, Frederick A. Fletcher, Eugene Maraskovsky, Terry Farrah, Douglas E. Williams, and Stewart D. Lyman. Immunex Research and Development Corporation, Seattle, WA 98101.

The ligand for the presumptive stem cell specific tyrosine kinase receptor FLT3 (also known as FLK-2) was obtained by direct expression cloning from a murine T cell clone P7B-0.3A4. The FLT3 ligand (FLT3L) is a transmembrane protein with similarities in both size and structure to SLF and CSF-1. A purified recombinant soluble form of the murine FLT3L was tested on pluripotent hematopoietic stem cells. Stem cells were cultured in the presence FLT3L alone, or in combination with IL-3 or SLF. After 5 days the cells present in each culture were counted and injected for day 14 CFU-S content determination. After 14 days the spleen colonies were counted and the cell equivalent of 0.5 femur of these primary recipient mice was injected into secondary recipient mice to determine the pre-CFU-S content of the cultures. After 5 days of culture in vitro, nucleated cellularity increased from 2-6 fold in growth factor stimulated cultures. No day 14 CFU-S were recovered from 5 day cultures with FLT3L alone. Low level maintenance (14%) of day 14 CFU-S was found with SLF + FLT3L. With IL-3 + FLT3L, 54% of the input day-14 CFU-S were recovered. However, the number of secondary day 14 CFU-S (pre-CFU-S) with FLT3L alone was 6.2-fold higher than the input number of pre-CFU-S and was also higher than in cultures with SLF or IL-3 with or without FLT3L. The data indicate that FLT3L alone is not capable of supporting the in vitro survival of day 14 CFU-S, but may be capable of supporting the survival and in vitro production of the more primitive pre-CFU-S.

H 111 HIGH EFFICIENCY DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO EITHER NEURONS OR SKELETAL MUSCLE IN VITRO. Jonathan H. Dinsmore, Douglas B. Jacoby, and Judson Ratliff. Diacrin, Inc., Bldg. 96, 13TH Street, Charlestown, MA 02129.

We have developed two conditions to induce differentiation of mouse embryonic stem (ES) cells reliably and at high efficiency into either neurons or skeletal muscle. The differentiated neurons express the neurotransmitter γ-aminobutyric acid (GABA) as well as many other neuron-specific proteins such as: neurofilaments, microtubule-associated protein 2 (MAP2), neuron-specific typeIII β-tubulin, and GAP-43. In the neuronal cultures, greater than 90% of the cells stain positive for neuron-specific markers, exhibit neuronal morphology, and fail to stain for the glial marker, glial fibrillary acidic protein (GFAP). The 10% of the population that lacks detectable expression of neuron markers stain positive for GFAP. Additionally, the differentiated neurons do not divide and exhibit a limited life span in culture, with neuronal cell death occurring after 5 days in culture. However, ES derived neurons survive a minimum of one month in vivo suggesting that the in vivo environment provides growth/survival factors not present in vitro. Thus, these ES derived neurons may serve as a useful in vitro screen for neuronal growth/survival promoting factors. When ES cells are induced to form skeletal muscle, we obtain greater than 95% pure skeletal myoblast cultures. The skeletal muscle myoblasts fuse and form myotubes that spontaneously contract in culture and stain for muscle specific markers such as muscle-specific myosin. Both the neurons and skeletal muscle cells are terminally differentiated and do not revert to the non-differentiated state when removed from conditions that promote differentiation. Currently, we are testing the in vivo function of these two cell types following transplantation.

H 112 IDENTIFICATION OF DOWNSTREAM TARGETS FOR FLK2/FLT3 RECEPTOR TYROSINE KINASE, Mercedes Dosil, Shulin Wang and Ihor R. Lemischka, Department of Molecular Biology, Princeton University, Princeton, New Jersey, NJ 08544-1014

Flk2/Flt3 is a receptor tyrosine kinase expressed in brain, placenta, testis and primitive hematopoietic populations. We have analyzed the mitogenic signalling potential and biochemical properties of Flk2/Flt3 in NIH 3T3 fibroblasts and interleukin 3 (IL3)-dependent Ba/F3 lymphoid cells. To circumvent the absence of known ligand, we used a chimeric receptor composed of the extracellular domain of the human colony-stimulating factor 1 (CSF-1) receptor and the transmembrane and cytoplasmic domains of Flk2/Flt3. Activation of the chimeric Flk2/Flt3 kinase with human CSF-1 leads to a transformed phenotype in transfected fibroblasts and abrogates IL-3 dependence in transfected Ba/F3 cells. These results indicate that Flk2/Flt3 has mitogenic potential in fibroblasts and in immature lymphoid cells. Furthermore, we have identified various SH2-containing proteins as participants of the Flk2/Flt3 signalling pathway and showed that this receptor tyrosine kinase exhibits specific substrate preferences. In order to explore the signal transduction mechanisms of hematopoietic stem cells we are now pursuing the identification of specific downstream targets for Flk2/Flt3 in primitive hematopoietic cells. To this end, we have generated a baculovirus vector to express the intracellular domain of Flk2/Flt3. The purified protein retains its kinase activity and can be autophosphorylated *in vitro*. Expression libraries from progenitor cell-enriched populations are currently being screened using the tyrosine-phosphorylated Flk2/Flt3 cytoplasmic domain as a probe.

H 113 EOSINOPHIL CYTOTOXICITY ENHANCING FACTOR: IMMUNOCYTOCHEMICAL DETECTION IN MYELOID PROGENITORS, HEMOPOIETIC CELL LINES AND RESTING AND ACTIVATED BLOOD CELLS. P. Xavier Elsas, R. C. Caminha, E. S. Maximiano, M. R. Pinheiro, H. Diamond, J. R. Lapa e Silva and M. I. Gaspar Elsas. Inst. Microbiologia/Fac. Medicina (UFRJ), Inst.Fernandes Figueira (FIOCRUZ), Rio de Janeiro, Brazil, 21941

In previous studies (Gaspar Elsas et al., Blood, 75, 2427) we had shown that monoclonal antibodies to the Eosinophil Cytotoxicity Enhancing Factor (ECEF), a heat resistant, eosinophil activating cytokine, detected a novel integral surface component of blood monocytes and T cells. In phorbol ester-induced differentiation assays, quantitative differences in the expression of this surface marker were associated with the ability of individual U937 histiocytic lymphoma cells to respond to PMA. In this study, we have used APAAP immunocytochemistry to investigate the expression of ECEF in progenitor cell populations purified from human bone marrow on discontinuous Percoll gradients, as well as in cells from GM colonies formed by these cells in agar. In parallel, we have detected this marker in myeloid cells isolated after collagenase digestion of Gartner-Kaplan (long term)-type cultures. We have also assayed for ECEF expression in peripheral blood monocytes before and after activation with LPS, and in T lymphocytes before and after activation with Concanavalin A. Finally, we have assessed ECEF expression in the HL60, KG1, KG1a, K562 and CEM cell lines, both in the cytoplasm and on the cell surface. The results, taken together, suggest that surface expression of ECEF epitopes is found 1) in bone marrow progenitors and mature and immature cells of the myelomonocytic lineage, and 2) in cell lines of the myelomonocytic phenotype. It is absent in the K562 (erythroid phenotype) cell line. Surface and cytoplasmic expression of ECEF are modulated during blood cell activation. While surface and cytoplasmic staining are usually associated, they could be dissociated in the CEM cell line, which has pure membrane expression. These findigs support the view that ECEF is a lineage-restricted marker modulated during hemopoietic cell development and activation.

H 114 **PARACRINE ACTIVITIES OF BRAIN DERIVED NEUROTROPHIC FACTOR IN HUMAN OLFACTORY NEURONAL PRECURSORS,** Fabrizio Ensoli*, Kaz Matsumoto*, Sharon Sickafuse*, Gabriella B. Vannelli@, and Carol J. Thiele*, *Pediatric Branch, National Cancer Institute, NIH, Bethesda, MD 20892;@ Dept. of Anatomy, University of Florence, Italy.

Throughout life olfactory sensory neurons are renewed from a population of dividing stem cells. Olfactory neurogenesis is both spatially and temporally controlled, however, little is known about the molecular mechanisms that regulate the activation, self-renewal and differentiation of immature olfactory neurons. To explore these issues, we have recently isolated, cloned and propagated *in vitro* primary long-term cell cultures from the human fetal olfactory neuroepithelium. These cells (FNC-A6 and FNC-B4) express neuronal and olfactory markers, are electrically excitable and one of them (FNC-A6) respond to aromatic chemicals. The results suggest that they originate from the olfactory "stem cell" compartment and are composed, at all times, of cells at different stages of maturation.

Our findings indicate that among several factors known to stimulate neuroblast survival and differentiation, these cells consistently express brain derived neurotrophic factor (BDNF) mRNA. In addition, biologically active BDNF was detected in the media conditioned (48 to 72 hrs) by the same cultures. Interestingly, these cells express NGF_R but do not express readily detectable levels of the trk mRNAs. Results of these studies and their implications in olfactory neurogenesis and the development of the olfactory system in humans will be discussed.

H 115 STRIATAL PRECURSORS TRANSPLANTED INTO THE CORTICAL VENTRICULAR ZONE GENERATES NEURONS WITH CORTICAL PHENOTYPES. G. Fishell. Laboratory of Developmental Neurobiology, The Rockefeller University, New York, N.Y., 10021.

One of the early steps in the regionalization of CNS is the subdivision of the forebrain into cortical and basal telencephalic ventricular zones (VZ). We have demonstrated that while neural progenitors migrate laterally within germinal zones of both cortex and basal telencephon, they do not cross the border between these VZs (Nature, **362**, 636, 1993). Molecular studies show restricted patterns of gene expression also respect the boundary between these germinal zones. To determine whether local signals within these regions of forebrain specify neural phenotypes, we performed heterotypic transplants of rat striatal VZ cells into rat cortical VZ at E15. Prior to transplantation, donor striatal precursor cells were labeled with the fluorescent lipophilic dye, PKH 26.

Two days after transplantation, at E17, labeled cells were seen migrating through the intermediate zone and into the cortical plate. Five days after transplantation, at E20, a large number of transplanted cells were positioned in cortical laminae. These cells had a neuronal morphology, with an apical dendrite and a descending axon that either entered the internal capsule or projected across the corpus callosum. Postnatally labeled cells had complex patterns of dendritic branching and numerous axonal boutons. Presently, we are analyzing the expression of cellular antigen markers to determine if transplanted cells express genes characteristic of cortical or striatal cells. These findings suggest that local signals within forebrain specify neuronal phenotypes characteristic of particular brain regions. (supported by NIH grant NS15429).

H 116 A NOVEL MUTATION AFFECTING THE LIGAND-BINDING REGION OF THE HUMAN KIT RECEPTOR.
Roger A. Fleischman, Dept. of Internal Medicine, Markey Cancer Center and VA Hospital, University of Kentucky Medical Center, Lexington, KY 40536
Binding of stem cell factor (SCF) to the Kit tyrosine kinase membrane receptor initiates a biochemical cascade that results in the proliferation, migration, or survival of stem cells in the hematopoietic, germ cell, and melanoblast lineages. Surprisingly, all ten of the missense mutations reported in *W/c-kit* mutant mice and piebald human kindreds have occurred in the cytoplasmic kinase domain, while none have been in the extracellular ligand binding domain. In contrast, missense mutations occur with equal frequency in both domains of the insulin receptor, a related tyrosine kinase. Recently, using genomic PCR we have identified a single-strand conformational polymorphism in exon 3 of human *c-kit* in one piebald kindred. Nucleotide sequence analysis of the cloned PCR product demonstrated heterozygosity for a novel missense mutation $Cys^{136} \rightarrow Arg$. $Cysteine^{136}$ is highly conserved in members of the Kit subfamily and is thought to participate in a disulfide bond required to form the second of five immunoglobulin-like loops comprising the extracellular domain. The relative rarity of other Kit extracellular domain mutations suggests that the second Ig-like loop may have an unusually important role in receptor function. In this regard, the second Ig-like domain appears to confer species-specificity to SCF binding by human-mouse hybrid receptors (Lev et al., Mol. Cell. Biol. *13*, 2224, 1993). Experiments are in progress to express this mutant receptor in vitro and examine its effect on ligand binding, receptor dimerization, and adhesion of hematopoietic cells to stromal cells.

H 117 IL-3, G-CSF, and Stem Cell Factor-Induced Ex Vivo Generation of Functionally Competent Myelocytic Cells
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Current peripheral blood progenitor cell transplantation protocols subject the recipient to an 8 to 10 day period of absolute neutropenia with associated morbidity, mortality, and expense. In this study we sought to exploit the proliferative and differentiative capacity of the hematopoietic stem and progenitor cell compartment in vitro to generate the mature cells required to abrogate this neutropenia. In order to determine the culture conditions necessary for this expansion CD34-positive cells were purified from peripheral blood progenitors mobilized with G-CSF, harvested by leukapheresis, and expanded for two weeks in various combinations of IL-3, IL-6, G-CSF, and Stem Cell Factor (SCF).

The optimal combination with regard to expansion of nucleated cells (simultaneous addition of 50ng/ml IL-3, G-CSF, and SCF) resulted in an average 773-fold expansion (range 493-1068). 75±10% of these cells were $CD11b^+$ and 86-91% were morphologically recognizable myelocytic cells. These cells exhibited normal phagocytosis and intracellular killing of *Staphylococcus aureus*. Substituting IL-6 for G-CSF in this combination resulted in considerably lower fold expansion (254±55-fold, range 125-431) and generated a population that was slightly less mature (i.e., 55±6% $CD11b^+$). This population also exhibited increased frequency of CFU-GM (6.3±0.2 per 1,000 cells; versus 1.1±0.1 per 1,000 cells with G-CSF). However, because of the difference in fold expansion the G-CSF, IL-3, SCF population contained a higher total number of progenitors. Similarly, sequential addition of late-acting factors to early-acting factors generated a less mature population with considerably lower fold expansion. These studies demonstrate that it is possible to generate clinically relevant expansion of functionally effective myeloid cells for use in abrogation of neutropenia using a relatively simple combination of growth factors.

H 118 IDENTIFICATION OF KERATIN 19 AS A NEW CELLULAR MARKER FOR EPITHELIAL STEM CELLS OF THE SKIN, Lucie Germain, Natalie Török, Pierrette Gaudreau, Marc Lussier and André Royal, Laboratoires des grands brûlés/LOEX, Hôpital du St-Sacrement, Québec, Qc, Canada, G1S 4L8 and Department of surgery, Laval university, Québec; Department of Pathology and Department of Medicine, Université de Montréal, Québec, Canada.

Skin stem cells have been localized in the bulge area of the hair follicles and at the tips of deep rete ridges of the epidermis using morphological or functional criteria. In the present study, the mouse skin was used as a model to look for a marker of cutaneous stem cells. A site-directed polyclonal antibody was raised in guinea pig against a synthetic peptide representing the segment 391-404 of keratin 19 (K19). This antibody was shown to react specifically with K19 by immunoblotting of total cell lysates of KLN 205 cells. Using this antibody, the distribution of K19 was studied in function of the hair cycle and the anatomic sites by immunofluorescence labeling. In newborn mice, K19 was present in cells of the external layer of the outer root sheat (bulge area) during hair follicle development. In adult mice, two patterns of labeling were observed among the various skin samples studied. For hairy anatomic sites (vibrissa, back, abdomen), K19 was present exclusively in cells of the bulge area, not detected in the epidermis. In contrast, for anatomic sites devoid of hair follicles (sole), K19 was present in the epidermis, at the tips of the rete ridges. Then, we determined if K19 was present in stem cells using a new double-labeling method. The functional assay labeling slow-cycling cells (^{3}H-thymidine, by autoradiography) was used in combination with K19 staining (by immunofluorescence) to co-localize them on tissue sections. The double-labeling showed that K19 containing cells are label-retaining cells. Taken all together, these results suggest that K19 is a specific marker for cutaneous stem cells.

This work was supported by the Medical Research Council of Canada, Fonds de la Recherche en Santé du Québec, Fondation des pompiers du Québec pour les grands brûlés et Fdn de l'Hôpital du St-Sacrement, Qc, Can.

H 119 DEVELOPMENTAL EXPRESSION OF PROTEIN TYROSINE KINASES DURING EARLY HEMATOPOIESIS IN MOUSE FETAL LIVER
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Protein tyrosine kinases (PTKs) are regulatory components in the signal transduction pathways of cell growth and differentiation. The involvement of these proteins in the function and maturation of hematopoietic cells has been shown both for cytoplasmic kinases (e.g. LCK, HCK, FYN, BLK, ATK/BPK) and growth factor receptors (e.g. c-KIT). During mouse embryogenesis hematopoietic cell commitment and differentiation takes place in the fetal liver from day 10 to day 17. Throughout this period several waves of different blood cell lineages emerge from this organ. With the aim of identifying the role of individual PTKs in early hematopoietic differentiation we have studied their expression at different time points of fetal liver development, which correlate with the predominant generation of a particular hematopoietic lineage. To this end we performed RT-PCR on RNA from fetal liver obtained from embryos at days 12, 15 and 17 of development. Primers corresponding to conserved regions within the catalytic domain were applied. Sequence analysis of PCR-products revealed differential expression of the receptor tyrosine kinases FLK-1, FLK-2, TEK, TIE-1, FGFR-4, Insulin receptor, PDGFRα, RYK, MEK4 and PTKFD16 on different days of development. The cytoplasmic kinases HCK, PTKFD15, the serine/threonine protein kinase PLK as well as JAK1 and JAK2, members of a new class of protein kinases, are also differentially expressed. The expression of these genes both in different hematopoietic cell lineages from fetal liver (characterized by the markers B220, 8C5, TER119, Thy1, Mac1) and progenitor cell populations (c-kit+, Sca1+, AA4.1+) will be presented.

H 120 A HIGHLY ENRICHED AND PHENOTYPICALLY HOMOGENEOUS POPULATION OF HEMATOPOIETIC STEM CELLS FROM MURINE BONE MARROW

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Using competitive repopulation as an assay for hematopoietic stem cell activity, we have developed a one-step enrichment procedure for murine hematopoietic stem cells. When experimenting with Hoechst 33342 dye, we observed an unusual phenomenon where, when Hoechst fluorescence was observed simultaneously at two wavelengths, multiple discrete populations are resolved. Testing various fractions of this complex staining pattern revealed that all of the stem cells stained uniquely in a region that was well separated from the rest of the cells of the bone marrow. This region represents approximately 0.1 % of the bone marrow. Furthermore, cells in this region are virtually homogeneous with regard to their cell surface expression of numerous markers (by antibody staining). The cell surface characteristics correlate well with the expression of markers previously tested by transplantation for marking stem cells, and are the following: $CD4^{low}$, $CD8^{neg}$, $CD5^{neg}$, $B220^{neg}$, Mac-1^{low}, Gr-1^{low}, c-kit^{pos}, $AA4^{low}$, Ter 119^{pos}, $M169^{pos}$, $CD43^{pos}$, $CD45^{pos}$. Human bone marrow also exhibits the unusual Hoechst staining pattern.

Using this highly enriched population from murine bone marrow, we are now attempting to separate cycling from quiescent stem cells, with a view to infecting the cycling cells with retroviral vectors. Preliminary analysis of the cell cycle status of these cells suggests that 1-3% of them are in $S\text{-}G_2M$.

H 121 PRETHYMIC EXPRESSION OF T CELL DIFFERENTIATION ANTIGENS IN THE MURINE FETAL LIVER: NOVEL SUBSETS OF LYMPHOID PRECURSOR CELLS, Regina Haars and Sophie Ezine, Génétique Moléculaire et Biologie du Développement, CNRS-UPR420, Villejuif, France 94801 and Différentiation thymique et physiologie des lymphocytes T, INSERM-U345, CHU-Necker, Paris, France 75015

T lymphocyte precursors are located in the fetal liver or the bone marrow. Whereas the presence of lymphoid precursors in the bone marrow has been widely demonstrated few reports exist on the fetal liver. Therefore, we have performed a systematic analysis of T cell differentiation antigen and T cell receptor (TCR) expression during ontogeny in the murine fetal liver at the molecular and cellular level. At the molecular level we find expression of the CD3 subunits as early as day 13 of gestation. The CD3ε subunit is expressed at the surface of fetal liver cells. These $CD3\varepsilon^{lo}$ cells are Thy1.2$^-$, FcγRII/III$^-$, TCRγ/δ$^-$, TCRβ$^-$, B220$^-$. No CD8 mRNA has been observed at any of the days tested, which is consistent with the absence of cell surface expression of the CD8 antigen. However, CD4 expression can be found on day 13 of gestation, the earliest day tested. From day 13 to 15 of gestation the CD4 gene is transcribed in form of a novel mRNA with the size of 3.5kb. This 3.5kb mRNA does give rise to $CD4^{lo}$ cell surface expression. The phenotype of the $CD4^{lo}$ cells has been determined to be FcγRII/III$^+$, Thy1.2$^-$, CD25$^-$, TCRγ/δ$^-$, TCRβ$^-$, B220$^-$

We are currently investigating the in vitro and in vivo developmental potential of the $CD3^{lo}$ and $CD4^{lo}$ fetal liver cells.

H 122 **COMPARISON OF THE EX-VIVO EXPANSION POTENTIAL FROM CD34$^+$, HLA-DR$^{+\ OR\ -}$, CD33$^{+\ OR\ -}$, AND CD38$^{+\ OR\ -}$ SUBSETS.** S. Heimfeld, R. Fei, J. Tsui, P. Thompson, and R. J. Berenson. CellPro, Inc., Bothell, WA.

Several investigators have suggested that the CD34$^+$ progenitor population can be further fractionated into more primitive and more mature cell compartments on the basis of co-expression of certain cell surface markers such as HLA-DR, CD33, and CD38. We have previously defined culture conditions which promote the ex-vivo expansion of unfractionated CD34$^+$ progenitor cells. In brief, those conditions involve the use of multiple cytokine combinations such as SCF, IL1, IL3, and IL-6 plus "serum-free" media supplemented with 5% human plasma. In this study we have examined the relative contribution that more primitive or mature CD34$^+$ progenitor cell subsets make to this overall expansion. The CD34$^+$ fractions were analyzed for increase in total cell numbers, CD34$^+$ cell numbers, CFU-GM, HPP-CFC and LTC-IC over a two week culture period. The results indicate that, on a per cell basis, the more primitive cell populations (CD34$^+$: DR$^-$, CD33$^-$, or CD38$^-$) give rise to 5-20 fold more CFU-GM, 5-10 fold more HPP-CFC and 5-10 fold more LTC-IC after two weeks of culture than do the corresponding more mature CD34$^+$: DR$^+$, CD33$^+$, or CD38$^+$ subsets. These results would suggest that ex-vivo expansion for clinical transplantation might be enhanced by further purifying subsets of more primitive CD34$^+$ cell. However, considering that the CD34$^+$: DR$^-$, CD33$^-$, or CD38$^-$ subsets represent only 1-5% of the total CD34$^+$ cells, in absolute terms the more primitive and more mature progenitor cells will contribute equal numbers of expanded CFU-GM.

H 123 IN VITRO DIFFERENTIATION OF EMBRYONIC STEM CELLS AS A MODEL OF HEMOPOIETIC DIFFERENTIATION, Cheryl D. Helgason and R. Keith Humphries, Terry Fox Laboratory, Vancouver, British Columbia, Canada, V5Z 1L3.

Embryonic stem (ES) cells, derived from the inner cell mass of murine blastocysts, can be maintained in a totipotent state in vitro by culture on embryonic fibroblasts or by growth in the presence of leukaemia inhibitory factor (LIF). Removal of LIF, followed by culture in suspension or in semi-solid media, has been shown to lead to differentiation into a variety of cell types including hemopoietic progenitors. We have characterized the differentiation of ES cells in suspension culture into various hemopoietic progenitors as a system to analyze patterns of gene expression during development and differentiation. Within 24-48 hours, the ES line CCE-J rapidly differentiates into cells capable of giving rise to multi-lineage hemopoietic colonies upon culture for 10-14 days in methylcellulose supplemented with appropriate growth factors including IL3, G-CSF, and erythropoietin. These progenitors are observed at a frequency of 80-325 per 10^5 cells. Erythroid-restricted precursors, observed within the same period of time, are present at a frequency of $1\text{-}3x10^3$ per 10^5 cells. Fluorescence activated cell sorting (FACS) was utilized to look for expression of hemopoietic surface antigens during the early stages of differentiation. Antibodies utilized included the lineage specific markers Ter 119 (erythroid), B220 (B cell), Gr-1 (granulocyte), Mac-1 (macrophages), Ly-1 (lymphoid), and the stem cell markers Sca-1 and AA4.1. Only the Sca-1 antigen, detected on up to 20% of the cells, was observed during the first two days of differentiation. RNA samples have been isolated from cells at various stages of differentiation. Northern blot analysis is being used to examine the expression of a number of genes relevant to hemopoietic differentiation. Genetic manipulation of undifferentiated ES cells, followed by in vitro differentiation, will facilitate elucidation of the roles of various genes in the generation of hemopoietic progenitors.

H 124 Self renewing and repopulating hematopoietic progenitors can be derived from embryonal stem cells *in vitro*. Nick Hole, Gerry Graham* & John D. Ansell. ICAPB and CGR, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, U.K. *CRC Beatson Laboratories, Garscube Estate, Glasgow G61 1BD, U.K.

Studies of the molecular control of hematopoiesis by genetic manipulation of the hematopoietic stem cell (HSC) are limited by the small numbers of *in vivo* HSC and difficulty in routine transgenesis; gene "knockouts" in HSC are not yet practicable. In contrast, transgenesis and gene "knockouts" are relatively facile in totipotential embryonal stem (ES) cells.

The differentiation of hematopoietic progenitors from ES cells *in vitro* was examined using both *in vitro* colony assays and *in vivo* reconstitution experiments. Using colony assays we have demonstrated the time course of appearance of the earliest detectable primitive hematopoietic progenitors following ES cell differentiation *in vitro*. These ES-derived hematopoietic progenitors possessed several phenotypic characteristics of HSC, including capacity for replating, sensitivity to inhibitors and possession of common surface antigens.

In a series of experiments, we have been able to demonstrate the ability of these ES-derived hematopoietic progenitors to rescue and repopulate lethally irradiated recipient mice. Differentiated ES cells were injected along with limiting doses of carrier spleen cells. After five months, over 25% of nucleated peripheral blood cells were ES-derived. ES-derived hematopoietic cells were found in both the myeloid and lymphoid compartments.

These data suggest that we have established a system for routine differentiation of HSC from ES cells *in vitro*. This system is currently being exploited in the study of the molecular control of hematopoiesis in normal and transgenic systems

Presenters I-Q

H 200 INTEGRIN EXPRESSION AND FUNCTION AND EPIDERMAL STEM CELLS IN VIVO,
Philip H Jones and Fiona M Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund, PO Box123, Lincoln's Inn Fields, London. WC2A 3PX, U.K.

It has long been hypothesised that human epidermis contains stem cells which maintain tissue integrity throughout adult life. These have been postulated to be a slowly cycling population of basal cells that occupy a specific anatomical niche. Human epidermal stem cells and transit amplifying cells can be isolated in vitro on the basis of differences in $\beta 1$ integrin expression and function. In vitro there is a log linear relationship between the level of expression of the $\beta 1$, $\alpha 2$ and $\alpha 3$ subunits in keratinocytes and their colony forming efficiency. We have investigated whether integrin expression and function can be used to delineate a stem cell population in vivo.

H 201 INFLUENCE OF NGF AND/OR GLUCOCORTICOID ON THE DIFFERENTIATION OF THE SYMPATHOADRENAL LINEAGE
Mitsuhiro Kawata, Masahumi Morimoto and Noriyuki Morita, Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, JAPAN

Sympathoadrenal progenitor cells (SPC) derived from neural crest differentiate into adrenal chromaffin cells under the influence of glucocorticoid, whereas SPC give rise to sympathetic neurons upon the action of NGF, indicating that the fate of these bipotential precursor cells is determined, at least in part, by the local milieu. We have tested the hypothesis of gradual narrowing of developmental potential of undifferentiated cells by culturing chromaffin cells from the postnatal(P)0, P5, P10, P15 and P25 days in conjunction with four combined treatments of NGF and dexamethasone(DEX); NGF+/DEX+, NGF+/DEX-, NGF-/DEX+, and NGF-/DEX-. As development proceeded, the effect of NGF on the process extension of chromaffin cells gradually diminished. The application of antisense oligonucleotide of glucocorticoid receptor protein into the culture medium induced the process extension of chromaffin cells even though they were treated with DEX, suggesting that the glucocorticoid effect on these cells is through its receptor. When we added antisense oligonucleotide of one of the cytoskeletal proteins, *tau*, into the culture medium, chromaffin cells retracted their processes, although these cells still expressed tyrosine hydroxylase activity.

H 202 BONE MORPHOGENETIC PROTEIN INDUCES CHONDROGENESIS IN MOUSE MESENCHYMAL STEM CELLS, Christina M. Klausmeyer, Shannon L. Pederson, John J. Rogers, and Henry E. Young, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.
Bone morphogenetic protein (BMP) has been used experimentally to induce cartilage and bone formation when implanted into extra-skeletal sites. These studies postulate that BMP induces cells at the implantation site to commit and differentiate into cells of the chondrogenic and/or osteogenic lineages. Recent studies have suggested that multipotent mesenchymal stem cells rather than fibroblasts compose the majority of the initial non-lymphocytic granulation tissue at a wound/implantation site and therefore would be potential recipients of BMP-inductive activity. This study was undertaken to examine the inductive potential of BMP on a clonal population of mouse pluripotent mesenchymal stem cells. Swiss-3T3-XYP mesenchymal stem cells were plated at various densities, treated with a 0.0 to 500 ng/ml concentration range of rh-BMP, and assayed for cellular proliferation, progression, and/or commitment. Immunochemical and histochemical stains were used to verify adult differentiated phenotypes. The results show that BMP is a inducer of chondrogenic commitment and expression in pluripotent mesenchymal stem cells. These results suggest that BMP may be used to stimulate chondrogenesis of mesenchymal stem cells at the wound/ implantation site and, in conjunction with non-fibrogenic differentiation factors, to stimulate tissue restoration rather than the obligatory scar tissue formation during tissue repair.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 203 CHARACTERIZATION OF HUMAN STEM AND PROGENITOR CELL EXPANSION IN BIOREACTORS, Manfred R. Koller, Brian Newsom, Clare E. Rogers, Gary Van Zant, Stephen G. Emerson, and Bernhard Ø. Palsson, Aastrom Biosciences, Inc. and University of Michigan, Depts. of Chem. Engineering and Medicine, Ann Arbor, MI.
Scale-up of hematopoietic cultures has been studied in perfusion bioreactors with bone marrow mononuclear cells (BM MNC), yielding expansion of cells, progenitors, and long-term culture-initiating cells (LTC-IC). We report here comparative results obtained with whole BM and CD34-enriched ($CD34^+$) populations. Each processing step resulted in enrichment of primitive cells, but was accompanied by a decreasing yield. For example, LTC-IC recovery from whole BM was 60% and 30% after density-separation and CD34-enrichment, respectively. The unabsorbed ($CD34^-$) fraction contained 17% of the LTC-IC, accounting for some of the observed decrease in yield upon CD34-enrichment. In initial experiments with $CD34^+$ cells in 24-well plates (fed every 48 hours), pre-formed stroma increased cell (2-fold increase), CFU-GM (5-fold), and LTC-IC (18-fold) output, suggesting that stromal elements are required for primitive cell maintenance. In bioreactors, whole BM, MNC, and $CD34^-$ cells were grown without pre-formed stroma because endogenous stromal development occurs with these cells (>100-fold increase in CFU-F). In contrast, $CD34^+$ cells were inoculated in bioreactors with pre-formed stroma. After 14 days, all cell populations significantly expanded. Erythropoietic and granulopoietic development occurred, as judged by changes in expression of CD71 and glycophorin A and of CD11b, CD15, and CD33, respectively. Flow cytometry also revealed a net expansion of primitive $CD34^+lin^-CD38^-$ cells. The culture yield (per ml of BM aspirate) was calculated from cell recovery and expansion data, thereby allowing comparison of results from the different procedures. For each ml of BM, the number of CFU-GM produced was 3.76×10^6, 1.42×10^6, 4.97×10^5, and 7.11×10^5 from cultures of whole, MNC, $CD34^-$, and $CD34^+$ cells, respectively. These results indicate that bioreactors expand stem and progenitor cells from whole BM as well as from the other starting populations. In addition, due to cell losses during density-separation and CD34-enrichment, cultures of whole BM resulted in the greatest yield of expanded cells per milliliter of BM aspirate. Furthermore, the final culture composition, with respect to lymphocytes, monocytes, granulocytes, and erythrocytes, was not significantly affected by the inoculum composition.

H 204 ODORANT RECEPTOR GENES EXPRESSED IN A HUMAN OLFACTORY NEUROBLAST CELL LINE

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Recent experiments with in situ RNA hybridization have revealed zonal expression of odorant receptor genes in the mammalian olfactory epithelium (Ressler et al., 1993 and Vassar et al., 1993). Within each expression zone, a neuron appears to select a restricted number of the receptor genes from a zonal gene set through a stochastic mechanism but not by positional information (ibid.).
It is yet to be answered: how many receptor genes will be selected in a given neuron; what mechanisms might underlie a stochastic choice; when and in what stage of development the choice of receptor genes is made.
To gain information on these problems, we analyzed normal human olfactory neuroblast cell lines (Vannelli G. B. et al., manuscript in preparation) and esthesioneuroblastoma (neuroepithelial-cell-derived neoplasm of the olfactory mucosa) cell line. The human olfactory neuroblast cell lines express the olfactory marker protein (OMP) characteristic of mature sensory neurons and odorant stimulation elicits a cAMP response of these cell lines (ibid.). Reverse transcription-PCR and RNase protection demonstrate that possible odorant receptor genes are expressed in the normal human olfactory neuroblast cell line but not in the esthesioneuroblastoma cell line. The ability of retinoic acid and NGF on the level of expression of these genes is currently under investigation. We will discuss whether a single olfactory neuroblast cell expresses more than one possible receptor gene, or whether this cell line is committed to a certain receptor gene subfamily but changes its receptor gene expression in a perpetual manner.

H 205 The use of β-galactosidase-gene-marked mouse hematopoietic stem cells to trace their fate and site of differentiation *in vivo*.
Eric Lagasse, Nobuko Uchida and Irving L. Weissman, Departments of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305

Hematopoietic stem cells (HSCs) are distinguished from other hematopoietic progenitors in the bone marrow by their unique ability to undergo multi-lineage (T, B, myeloerythroid, etc) differentiation and self-renewal. In *in vivo* HSC transfer studies, isolated individual HSCs had to circulate in the blood and seed hematopoietic microenvironments. Repopulating outcomes depend on the specific microenvironments where individual HSCs land. Injection of donor hematopoietic cells from one mouse strain into a congenic host strain allows the differentiated progeny of HSC to be examined *in vivo*. The Ly-5 antigen (T200) is expressed on the surface of most hematolymphoid cells, and Ly-5 allelic differences can be detected by monoclonal antibodies specific for either the Ly-5.1 or the Ly-5.2 allele. Cells from the Ly-5.1 strain (donor type) can be transferred into Ly-5.2 congenic mice (host type) without crossing the MHC barrier. However, some lineages (e.g. erythroid) lose Ly-5 expression during differentiation, and therefore it is difficult to trace the fate of Ly-5-marked HSCs in those lineages.

To resolve this problem, we have backcrossed C57-BL/Ka-Thy-1.1 strain with ROSAβ-geo26, originally characterized by Friedrich and Soriano (Genes & Development, 5:1513-1523, 1991) to derived mice with ubiquitous expression of the β-galactosidase gene (C57-BL/Ka-Thy-1.1-βgeo26). HSCs from this strain can be traced by either using fluorescein di-β-D-galactoside and FACS, or histochemically *in situ*. Whole bone marrow or purified $Thy\text{-}1.1^{lo}$ $Lin^{-/lo}$ $Sca\text{-}1^+$ cells from this strain were transplanted into lethally irradiated Ly-5 congenic recipients. When we examined spleens of the radiation chimeras at day 7 day post transplantation, $\beta\text{-galactosidase}^+$ / $Ly\text{-}5^-$ donor-marked cells were detected. We hope that this new congenic mouse system allow us to elucidate the lineage fate of hematolymphoid and other putative bone marrow-derived cells (e.g. microglia), and the microenvironments for their differentiation.

H 206 DIFFERENTIAL EXPRESSION OF HOX A CLUSTER GENES IN CD34+ PRIMITIVE SUBPOPULATIONS OF HUMAN BONE MARROW CELLS. Guy Sauvageau, Corey Largman, H. Jeffrey Lawrence, Peter Lansdorp, Donna Hogge, Connie, E. Eaves, Wieslawa Dragowska, and R. Keith Humphries. Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, B. C., Canada and VA Medical Center, San Francisco, CA. 94121

The HOX genes, first identified in Drosophila, are now recognized as key determinants of mammalian development. Recent evidence of HOX gene expression in leukemic cell lines, their involvement in chromosomal translocations in human leukemia, and our own preliminary analysis of expression of HOX genes in normal CD34+ cells have suggested that these genes may have important roles in the growth and differentiation of primitive hemopoietic cells. To characterize in detail the pattern of HOX gene expression in early hemopoietic cells, we have isolated by FACS subpopulations of CD34+ bone marrow cells highly enriched for long term culture-initiating cells (LTC-IC) or clonogenic myeloid or erythroid progenitors respectively and generated amplified cDNA from each population using an improved RT/PCR technique which enables the generation of extended length and representative cDNA from fewer than 1000 cells. Subsequent amplification of cDNA using degenerate primers to homeodomains and sequencing of products revealed expression of at least 7 of 11 known cluster A HOX genes. The relative level of expression of 3 of these (HOX A10, A7, and A4) has now been explored in detail by Southern analysis of the initial amplified total cDNA from each subpopulation using homeodomain free probes. HOX A10 and A7 expression was found to be essentially constant in LTC-IC vs clonogenic progenitor enriched populations. HOX A4 expression was at least 10 fold higher in the subpopulations containing LTC-IC than in subpopulations enriched for myeloid and erythroid progenitors and devoid of LTC-IC. Analysis of the remaining members of the A cluster are now in progress. These data not only suggest that HOX A cluster genes are widely expressed in early hemopoietic CD34+ subpopulations, but, that at least one of HOX A cluster is differentially expressed making this gene a potential determinant of hematopoietic stem cell function.

H 207 A COMMON PRECURSOR FOR NEURONS, TYPE 1 ASTROCYTES AND O-2A PROGENITORS EXISTS IN THE POSTNATAL SUBVENTRICULAR ZONE. Steven W. Levison and James E. Goldman, Dept. of Pathology, Columbia University , NY, NY 10032, and Dept. of Neuroscience and Anatomy, PSU School of Medicine, Hershey, PA 17033.

We have used retroviral mediated gene transfer and immunolabeling to follow the fates of cells in the postnatal subventricular zone (SVZ) of the rat. We recently demonstrated that *in vivo* a single cell is capable of producing both protoplasmic astrocytes and oligodendrocytes. To determine their developmental potential, SVZ cells were labeled *in situ* by stereotactic injection of retrovirus. Twenty four, or four, hours later the region containing the SVZ was excised, dissociated and cultured. One week later the composition of each clone was established using enzyme histochemistry for the reporter gene, cell morphology and antigen expression. When cells were maintained in medium supplemented with 5% FBS, 50% of the clones were homogeneous containing cells of either the type 1 astrocyte or the O-2A lineage, and approximately 50% of the clones contained cells from both these lineages. By maintaining the SVZ cells in medium supplemented with 20% horse serum, a greater number of retrovirally labeled cells survived, and approximately 60% of the clones contained cells of glial lineages as well as cells classified as neurons based on morphology and antigen expression (MAP-2+/A2B5+/O4-/GFA-). Retrovirally labeled neurons were not evident in clones that were maintained in medium lacking horse serum, although some neurons were present in these cultures. On average, the neuron-containing clones contained 4 neurons per clone, although 19 neurons were documented in one clone. Neuron-containing clones were larger than those that lacked neurons. Finally, mixed glial lineage clones have also been observed in cultures established from postnatal day 14 SVZ when the cells were maintained in medium supplemented with 5% FBS. These results indicate that multipotential neural precursors persist in the postnatal germinal zone, arguing against an early commitment of precursors to specific lineages during the development of the rat cerebral cortex.

H 208 A STUDY OF HIGH DOSE CYCLOPHOSPHAMIDE, CTCb (CARBOPLATIN, THIOTEPA, CYCLOPHOSPHAMIDE) FOLLOWED BY STANDARD DOSE ADRIAMYCIN IN PATIENTS WITH METASTATIC BREAST CANCER: PRELIMINARY RESULTS. Magalhaes-Silverman M, Rybka WB, Lembersky B, Kiss J, Bloom E, Lister J, Pincus SM, Selvaggi KJ, and Ball ED. Bone Marrow Transplant Program Pittsburgh Cancer Institute University of Pittsburgh, Pittsburgh, PA 15213.

Between October 1992 and September 1993, ten patients (pts.) with metastatic breast cancer (MBC) were enrolled in a therapeutic trial consisting of three phases. During the first phase, pts. received high dose cyclophosphamide at 5000mg/m² x 1 dose with G-CSF at 5μg/kg which was followed by peripheral blood stem cell collection. The second phase consisted of high dose cyclophosphamide (6000mg/m²), thiotepa (500mg/m²) and Carboplatin (800mg/m²) (CTCb) with blood progenitor cell support. During the third phase, pts. received Adriamycin 60mg/m² x 1 dose every 3 weeks for 3 cycles. INCLUSION CRITERIA: age < 65; measurable disease; no prior chemotherapy (CT) for metastatic disease; ER negative; if ER positive rapidly progressive visceral disease or hormonal manipulation failure. PTS. CHARACTERISTICS: median age 37 yrs. (range 26-50); med Zubrod PS 1 (0-2); disease sites: visceral 5/10, bone 7/10, soft tissue 1/10; 8/10 had prior adjuvant CT. RESULTS: 10 pts. completed the first phase; 8 pts. the second and 6 pts. the third phase. Five pts. are evaluable for response. One pt. achieved CR and 4 PR. TOXICITY: during CTCb the median times to neutrophil $\geq$ 5 x 10^9/L and platelets $\geq$ 20x10^9/L were 9d (range 8-12) and 8d (range 7-11). One pt. had E. coli bacteremia. During the third phase 3 episodes of neutropenic fevers were observed (2 culture negative, 1 E. coli). No toxic deaths were observed. CONCLUSION: This dose-intensive sequential approach for MCB is feasible with acceptable toxicity. Accrual into this trial continues.

H 209 ISOLATION OF AVIAN MESENCHYMAL STEM CELLS FROM MULTIPLE CONNECTIVE TISSUE SITES, Matthew L. Mancini, Robert P. Wright, Jennifer C. Smith, John J. Rogers, Paul A. Lucas, and Henry E. Young, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.

Previous studies have noted the presence of mesenchymal stem cells located within the connective tissue matrices of avian skeletal muscle, skin, atria, and ventricles. Clonal analysis coupled with dexamethasone treatment revealed the presence of multiple populations of mesenchymal stem cells composed of both lineage-committed stem cells and pluripotent stem cells. As a follow-up, this study was undertaken to assess the distribution of these mesenchymal stem cells within various connective tissue-associated regions of the body. Day 11 chick embryos were divided into 26 separate regions based on associated connective tissue components. Heart, skeletal muscle, and dermis were included as comparative control tissues. Cells were isolated using conditions optimal for the isolation, cryopreservation, and propagation of avian mesenchymal stem cells. Cell aliquots were plated at 2x10^4 cells/well in 24-well 1% gelatinized tissue culture plates, incubated with a 10^{-10} to 10^{-6}M concentration range of dexamethasone and examined for pluripotentcy. Immunochemical and histochemical staining procedures were used to verify differentiated morphologies. As noted, four recurring phenotypes appeared , i.e., skeletal muscle myotubes, fat cells, cartilage nodules, and bone nodules. These results suggest that mesenchymal stem cells are located within any region, tissue, and/or organ having a connective tissue component.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 210 PURIFIED MURINE STEM CELLS: THEIR COLONIZATION POTENTIAL AND THE EXPRESSION OF HEMATOPOIETIC GROWTH FACTOR RECEPTOR GENES DURING IN VITRO DIFFERENTIATION, Giovanni Migliaccio, Gian Carlo Mancini, Yajuan Jiang, Eishi Ashihara, Damianos Sotiropoulos, Anna Rita Migliaccio, and John W. Adamson. The New York Blood Center, New York, NY, USA and Istituto Superiore di Sanità , Rome, Italy

We have analyzed the reconstitution potential and the expression of hematopoietic growth factor receptor genes of pluripotent murine hematopoietic stem cells (HSC) purified from normal bone marrow on the basis of several criteria including their ability not to retain rhodamine 123 (Rho^-). Rho^+ cells were analyzed as controls. Rho^- (10^2-10^4 cells), but not Rho^+, permanently and completely reconstituted W/W^v mice 4 months after transplantation. The ability of the cells to proliferate in vivo or in vitro was analyzed by transplanting marrow cells (10^5-10^6 cells/mouse) from reconstituted primary recipients 7 months following transplantation, or Rho^- cells cultured for 5-10 days in the presence of stem cell factor (SCF) and IL-3. Secondary recipients which received the reconstituted marrow equivalent to ≤1 original Rho^- cell were completely reconstituted, indicating Rho^- cells have a high proliferation potential in vivo. Reconstitution also was observed with Rho^- cells cultured in vitro for 5 days, but not for 10 days. Dilution experiments indicated that HSC were only maintained in culture. We also investigated by RT-PCR the expression of a number of hematopoietic growth factor receptor genes in purified HSC at the outset and after stimulation for 3 days in serum-free liquid culture with SCF and IL-3. Receptor gene expression for early-acting growth factors (IL-3, GM-CSF) was seen in both Rho^- and Rho^+ cells while genes for receptors for late-acting growth factors (Epo, G-CSF and M-CSF) were differentially expressed. Epo-R was expressed in Rho^+ cells at the outset and was induced in Rho^- cells by day 3 after stimulation with SCF and IL-3. G-CSF-R and c-fms were both in Rho^+ and in Rho^- cells at the outset and their expression increased in culture of Rho^+ cells and decreased in culture of Rho^- cells. These experiments indicate the high proliferative potential of Rho^- cells in vivo and suggest that expression of the Epo-R gene could represent a molecular marker of (the human) HSC.

H 211 FUNCTIONAL ANALYSIS OF HEMATOPOIETIC PROGENITORS OF W^{41}/W^{41} AND $W^{42}/+$ GENOTYPE, Cindy Miller and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, B.C. V5Z 1L3 Canada.

In mice, mutations at the Dominant White Spotting (W) locus can cause macrocytic anemia, a reduction in tissue mast cells and other hematological defects. For example, primitive hematopoietic cells from W/W^v mice are unable to generate macroscopic spleen colonies in irradiated recipients and cells of +/+ origin can form such colonies and competitively repopulate unirradiated W/W^v mice. Such observations together with the more recent identification of the W (c-kit) gene product as a cell surface receptor for Steel factor (SF) and demonstration of c-kit expression on repopulating cells, have been interpreted as evidence of a physiologically important role of c-kit in primitive hematopoietic stem cell regulation. To examine the importance of c-kit function at different levels of hematopoietic cell development we have initiated studies of the effect of W^{41} and W^{42} mutations on the detection of a relatively late type of erythroid progenitor (Day 3 BFU-E) in vitro, and on the detection of more primitive hematopoietic cells in both short term (CFU-S) and longer term (CRU) in vivo repopulation assays.

Bone marrow cells derived from mutant W^{41}/W^{41} or $W^{42}/+$ animals, when compared to +/+ littermates, did not differ significantly in their capacity to form small erythroid bursts after 3 days in semi-solid medium containing optimal levels of erythropoietin (3U/ml). However, the ability of SF to enhance the formation of these colonies at suboptimal eyrthropoietin concentrations was marginal in assays of cells from the mutant mice. In vivo studies indicated that W^{41}/W^{41} and $W^{42}/+$ mice contained cells detectable both as Day 9 and Day 12 CFU-S albeit at reduced frequencies (about 2-fold less relative to +/+ controls). In addition, the mutant CFU-S produced slightly smaller colonies at both time points. Longer term repopulation assays indicated that stem cells from adult W^{41}/W^{41} mice could competitively repopulate irradiated +/+ recipients but this required the transplantation of approximately 10x more cells than from +/+ mice. These studies set the stage for future experiments to analyze the effects of these W mutations on CRU self-renewal and the identification of factors that may complement SF functions at different stages of hematopoietic cell differentiation.

H 212 *Abstract Withdrawn*

H 213 TOWARDS IDENTIFICATION OF THE FLK2-RECEPTOR TYROSINE KINASE LIGAND, Kateri A. Moore, *Larry Witte, and Ihor R. Lemischka, Princeton University, Princeton, N.J. 08540, *Imclone Systems Inc., New York, New York 10014

Class III receptor-type tyrosine kinases (RTK) include receptors for hematopoietic growth factors. Examples are c-fms, the receptor for macrophage colony stimulating factor and c-kit, the receptor for stem cell factor. Flk2, also known as flt3, is included in this class. Flk2, identified in this laboratory, was isolated from an enriched population of hematopoietic stem/progenitor cells and has been shown to be differentially expressed in the hematopoietic hierarchy in addition to brain, placenta and testis. Given this, the role of flk2 and the function of its cognate ligand is of potential importance in hematopoiesis. Our attempts to isolate the ligand for flk2 involve two different but complementary expression cloning strategies; 1) autocrine loop cloning with a eukaryotic phage expression vector and 2) using a fusion protein consisting of the flk2 extracellular domain joined to the human IgG1 Fc domain to detect cloned cDNA's. We have constructed cDNA libraries from newborn brain in lambda pCEV-27 and a fetal liver stromal cell line in pSVsport. The stromal cell line was selected from a panel of many made in this laboratory for its unique properties of stem cell maintenance and its ability to specifically bind flk2 fusion protein. The stromal cell line cDNA library has been transfected into cos-1 cells and screened with the chimeric fusion protein. Specific binding has been detected and sub-pools are being re-analyzed. Transfection of the neonatal brain cDNA library into flk2 expressing NIH-3T3 cells yielded 27 foci which demonstrated serum-free growth after G418 selection. The plasmids were excised from genomic DNA from these foci and the resultant transformants were pooled for each focus. Plasmid DNA from each focus was transfected into both the parental 3T3's and the flk2-3T3's, again selected in G418 and replated into serum-free media (SFM). Of the 27 different transfectants one formed foci in flk2-3T3's only. These foci are being examined further by re-transfection of individual plasmids. Candidate clones will also be transfected into cos-1 cells for binding and competition studies with the flk2 fusion protein in addition to co-transfection with flk2 receptor plasmid followed by assay for transforming potential in SFM. Together these strategies should lead to the eventual identification of the flk2 ligand which will allow determination of the biological involvement of this receptor in hematopoiesis.

H 214 THE MYOGENIC STEM CELL - DOES IT EXIST?

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Myoblast transplantation, proposed as a treatment for inherited myopathies such as Duchenne Muscular dystrophy, would be most efficacious if the injected myoblasts were able to repopulate progressively larger volumes of muscle with increasing time. For this purpose, a myogenic stem cell, able to reconstitute the entire myogenic compartment of the host muscle, would be of value. We have produced evidence for such a myogenic stem cell by making use of a mouse transgenic for an inducible construct encoding a thermolabile mutant of the SV40 large T antigen. Myogenic cells from this mouse can be cloned extensively under conditions where the T antigen is expressed and active and, on return to normal conditions, will differentiate normally. As well as forming muscle *in vivo*, these cells formed, albeit rarely, quiescent cells which gave rise to myogenic cells on subsequent culture of the injected muscle. These extracted cells have, so far, been passaged through 3 generations of irradiated mdx nu/nu mouse muscles, giving rise to muscle of normal appearance and extractable myogenic cells which behave like the original clone *in vivo*.

We are now investigating further the behaviour of these putative myogenic stem cells *in vivo*.

H 215 HEMATOPOIETIC STEM CELLS CAN BE DISTINGUISHED FROM TRANSIENT HEMATOPOIETIC PROGENITORS BY MAC-1 EXPRESSION,

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Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{hi} cells, representing 0.05% of C57BL/Ka-Thy1.1 bone marrow, are highly enriched for hematopoietic stem cells; however, the functional reconstituting activity of this fraction is heterogeneous, containing both long term and transient multipotent hematopoietic reconstitution. Transient multipotent reconstitution is typically characterized by declining levels of B and T cell reconstitution, and the disappearance of donor derived myeloid cells between 6 and 8 weeks after reconstitution. In an effort to resolve the transient and long term reconstituting fractions within the Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{hi} population, the progenitor activities associated with each lineage marker within the Thy-1.1^{lo}Sca-1^{hi} fraction were assayed. The only lineage markers associated with progenitor activity were Mac-1 and CD4. The Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{hi} population was resolved into three subpopulations: Thy-1.1^{lo}Sca-1^{hi}Mac-1^{-}CD4^{-}Lin^{-}; Thy-1.1^{lo}Sca-1^{hi}Mac-1^{lo}CD4^{-}Lin^{-}; and Thy-1.1^{lo}Sca-1^{hi} Mac-1^{lo}CD4^{lo}. The Thy-1.1^{lo}Sca-1^{hi}Mac-1^{-}CD4^{-}Lin^{-} population is highly enriched for long term reconstituting hematopoietic stem cells. 25 Thy-1.1^{lo}Sca-1^{hi}Mac-1^{-}CD4^{-}Lin^{-} cells long term reconstituted 5 of 7 lethally irradiated mice. Both progenitor populations expressing low levels of Mac-1 were highly enriched for transient reconstitution activity. Nine of 10 lethally irradiated mice exhibited transient reconstitution by 30 Thy-1.1^{lo}Sca-1^{hi}Mac-1^{lo}CD4^{-}Lin^{-} cells. No progenitor activity was observable from cells coexpressing Thy-1.1^{lo}Sca-1^{hi} and either Gr-1, CD5, B220, CD8, or high levels of CD4. Thus lineage marker expression is correlated with functional activity among Thy-1.1^{lo}Sca-1^{hi} hematopoietic progenitors.

H 216 NEURAL STEM CELLS IN THE SUBEPENDYMA OF THE ADULT MAMMALIAN FOREBRAIN,

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A population of stem cells isolated from adult mammalian brains has the capacity to proliferate and differentiate *in vitro* in the presence of EGF. The proliferating cells form spheres and the differentiated progeny of these cells *in vitro* are neurons and glia. The adult brain is mitotically quiescent *in vivo* with the exception of the subependymal region lining the lateral ventricles in the forebrain. This region contains a subpopulation of constitutively proliferating cells with a cell cycle time of 12.7 hours. The cells elicit a steady-state mode of proliferation whereby one progeny undergoes cell death and the other continues to divide. We investigated whether the stem cells isolated *in vitro* originated from the subependymal layer *in vivo*. Dissection and subsequent culturing of adult hippocampus, cortex, striatum alone and striatum including the lateral wall of the lateral ventricle resulted in the formation of spheres only when dissections included cells from the subependyma. Stem cells *in vitro* are responsive to EGF and positive for nestin (a neuroepithelial stem cell marker). We also observe EGF-receptors and nestin expression in the proliferating subependymal population *in vivo*. To ask whether the normally proliferating subependymal cells are the only source of stem cells *in vitro* or whether a separate, normally quiescent, subependymal cell could serve as the ultimate source of stem cells, we carried out tritiated-thymidine kill experiments. Animals were injected with high doses of radioactive thymidine for the duration of the cell cycle at intervals less than S-phase to kill all the normally proliferating cells. The proliferative population was assessed at 1, 2, 4, 6, 8, and 12 days post-kill by injecting bromodeoxyuridine (BrdU) prior to sacrifice. One day post-kill, the proliferating population was 10% of controls and by 8 days, the proliferating population was back to control values. If the replenished population is due to the recruitment of normally quiescent cells into the proliferative mode, we hypothesized that a second series of radioactive thymidine would kill the newly recruited population. Indeed, the maximal depletion of proliferating cells is maintained at 50% of controls (8 days post-kill) when the second kill is done 1 or 2 days after the initial kill. When the second kill is done on day 4, the proliferating population returns to control values 8 days later. Taken together this data suggests a time window of recruitment of normally quiecent cells into the proliferative mode (the first two days after the initial kill), and that by 4 days the quiescent subependymal cells are no longer being recruited. Our data suggests the presence of a quiescent stem cell in the adult mammalian subependyma, which gives rise to proliferative progenitors *in vivo* and possibly *in vitro*.

H 217 DICISTRONIC TARGETING VECTORS: REPORTERS AND MODIFIERS OF ENDOGENOUS GENE ACTIVITY.

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To better understand the role of localised and cell-type specific expression of regulatory molecules governing stem cell proliferation and differentiation, we have developed high efficiency "gene trap" type targeting vectors to report and modify expression of endogenous candidate genes. Integration of the promoterless targeting vector into a transcriptionally active gene intimately couples lacZ/neomycin fusion gene (ßgeo) expression with target gene transcriptional and post-transcriptional regulatory elements through the generation of a fusion transcript. As translation of the ßgeo cassette is mediated via an Internal Ribosome Entry Site (IRES) and therefore independent of context in the fusion transcript, design and construction of targeting vectors is greatly simplified. Potential restrictions associated with non-IRES based gene traps including the generation of fusion proteins and reinitiation of translation (stop/start) are eliminated. Vectors can be conveniently designed either to report normal gene expression by simple insertion, or altered expression resulting from defined modifications at the locus.

Differentiation Inhibiting Activity/Leukaemia Inhibitory Factor (DIA/LIF) is a pleiotropic cytokine which suppresses differentiation of ES cells *in vitro* and has been implicated in a variety of developmental and physiological processes *in vivo*. We have modified the endogenous DIA gene locus using a variety of constructs designed to analyse the expression profile of the DIA gene at the cellular level both *in vitro* and *in vivo*, and to examine the functional significance of the long (3 kb) 3' untranslated region. LacZ staining patterns reveal cell type specific expression in differentiated cultures of targeted cell lines. Chimaeric mice have been generated for *in vivo* analysis.

H 218 MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1; MCAF, mJE) IS CHEMOTACTIC FOR CULTURED TRIPLE NEGATIVE ($CD3^-$,$CD4^-$, $CD8^-$, $THY1.1^+$) T LYMPHOCYTES. William E. Munger, Thomas Cox, Courtney Beers, Douglas Pat Cerretti, Charles Rauch, Anthony Namen, Barrett J. Rollins*, and June Eisenman. Immunex Corp., Seattle, WA 98101 and *Dana-Farber Cancer Institute, Boston, MA 02115

The signals which govern how hemapoietic progenitor cells traffick through various tissues as they differentiate remain poorly understood. We have examined early murine T cells at the triple negative (TN) stage for chemotactic and chemokinetic abilities. The TN were derived from day 13 fetal liver and cultured long term in vitro under Whitlock-Witte-like conditions. Using Costar transwells (3μ diam. pores) we tested a panel of chemokines including huGro, huIL8, muMIP1α, muMIP1β, Rantes, muIP-10, and muMCP-1. Only MCP-1 induced migration above background. Migration plateaued by 60 minutes (e.g., 18% of TN migrated to 50 ng/ml MCP-1 vs. 1% background). Checkerboard analysis (see table) revealed that most of the migration was chemotactic rather than chemokinetic in nature.

MCP-1 (ng/ml)		Upper Well 0	1.5	6	24
Lower Well	0	1.0*	1.0	1.0	1.0
	1.5	1.8	1.5	0.3	0.7
	6	3.6	1.7	0.6	1.0
	24	8.3	3.6	1.9	1.9

*Chemotactic Index (%MCP-1-induced migration/%Control migration)

More mature T cells in spleen, lymph node and thymus did not respond to MCP-1. Thus, MCP-1 may facilitate T cell development by guiding tissue localization of TN during a transient MCP-1-responsive stage.

H 219 **HARVEST OF OSTEOBLASTIC PROGENITORS FROM HUMAN BONE MARROW BY ASPIRATION**

George Muschler, Akira Hyodo, Vladimir Scerbin, Department of Biomedical Engineering and Orthopaedic Surgery, The Cleveland Clinic Foundation, Cleveland, Ohio 44195.

Osteoblastic progenitors can be assayed in bone marrow using colony forming units (CFUs) and expression of markers of osteoblastic differentiation, such as type I collagen, alkaline phosphatase (AP), osteocalcin and matrix mineralization. Harvest of these cells may have clinical applications for bone grafting applications and for cell based therapies. This report characterizes the harvest osteoblastic CFUs from adult human bone marrow aspirates and begins to assess variables which may influence osteoblastic CFU yield.

METHODS Bone marrow samples (2 cc samples) were aspirated from the iliac crest in thirty patients, 22 normal bone marrow donors (mean age 34, 8 to 69) and 8 otherwise normal patients undergoing elective surgery for osteoarthritis (mean age 64, 44 to 80). Heparinized aspirates were centrifuged. The buffy coat was harvested and resuspended in 5 ml alpha-MEM. Nucleated cells were counted. 10^6 nucleated cells from each aspirate were cultured in six 10 cm^2 wells using alpha-MEM, 10 % FCS, Dexamethasone (10^{-8} M). Colonies expressing AP were counted manually at 9 days following in situ staining for AP. The original buffy coat preparation were analyzed using an automated cell counter and WBC/ml, WBC/RBC ratio, and % lymphs in the buffy coat were assessed as independent variables.

RESULTS Osteoblastic CFUs were harvested from all but two patients. Patient age was negatively correlated with the yield of alkaline phosphatase producing CFUs (p= .023, Spearman Rank). However, a disease effect associated with osteoarthritis could not be ruled out since comparison of normals (mean 51, range 3 to 103) to osteoarthritics (mean CFU 26, range 0 to 60) was also significant (p=.04, Mann-Whitney). WBC/ml was positively correlated with CFU (p= 0.016, Spearmann). %lymphs and WBC/RBC ratio were not correlated with CFUs.

CONCLUSIONS The yield of osteoblastic CFUs from human bone marrow aspirated appears to decrease with age. Even so, osteoblastic CFU can be obtained from almost all donors.

H 220 INTERLEUKIN-4 CAN REVERSE THE INHIBITORY EFFECT OF INTERLEUKIN-3 ON B-LYMPHOPOIESIS IN CLONAL CULTURES OF MURINE LYMPHOHEMATOPOIETIC PROGENITORS. Steven Neben*, Debra Donaldson*, Lori Fitz*, James Calvetti*, Katherine Turner*, Fumiya Hirayama# and Makio Ogawa#. *Genetics Institute, Cambridge, MA 02140 and #Department of Medicine, Medical University of South Carolina and #Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29401

We have previously described a two-step methylcellulose culture system in which individual primitive progenitors from 5-fluorouracil treated mice can be demonstrated to have both myeloid and B-lymphoid differentiation capacity. Highly enriched $Lin^-Sca^+FU_{2d}BM$ cells were plated in methylcellulose in the presence of steel factor (SF) and pokeweed mitogen stimulated spleen conditioned medium (PWM-SCM). Primary mixed myeloid colonies were replated after 8-11 days into secondary cultures containing SF and IL-7, yielding secondary $B220^+sIgM^-$ pre-B cell colonies. A number of growth factors, including IL-6, IL-11, G-CSF, and IL-12 were capable of substituting for PWM-SCM to support the B-lymphoid potential of primary colonies. Interestingly, B-lymphoid potential is not supported in SF + IL-3, or in SF + IL-3 plus any single growth factor (IL-1 to IL-12, GM-CSF, G-CSF, Epo, LIF, TNFα, TGFβ, IFNγ, or IGF-1) tried, but is supported in SF + IL-3 + 5% PWM-SCM. Experiments aimed at identifying the factor(s) in PWM-SCM that reverse the inhibitory effects of IL-3 on B-lymphoid potential gave the following results: 1) the activity co-purified with IL-4 through several steps of biochemical fractionation; 2) IL-4 antibody neutralized the activity in PWM-SCM and 3) combinations of IL-4 + IL-6 or IL-4 + IL-11, but not IL-4 alone, could reverse the inhibitory effects of SF + IL-3 on B-lymphoid potential in primary cultures. These studies suggest that commitment of primitive lymphohematopoietic progenitors to the B cell lineage may be tightly regulated by multiple growth factors, including IL-3, IL-4, IL-6, and IL-11.

H 221 ISOLATION OF GENES SPECIFICALLY EXPRESSED IN RT4-E, A NEURONAL CELL TYPE, AND NOT IN RT4-AC, A STEM CELL LINE BY SUBTRACTIVE HYBRIDIZATION AND DIFFERENTIAL SCREENING, Risa Ohkawa and Noboru Sueoka, Molecular, Cellular and Developmental Biology Department, University of Colorado, Boulder, CO 80309-0347

Our work focuses on studying the molecular mechanisms of cell determination and differentiation using the RT4 cell lines. The RT4 family of cell lines was derived from a rat peripheral neurotumor. The stem cell line RT4-AC repeatedly gives rise to three distinct derivative cell types RT4-B, RT4-D, and RT4-E. This conversion process is accompanied by the segregation of properties; the stem cell line expresses both neuronal and glial characteristics while the derivative cell types express either neuronal or glial characteristics. RT4-B and RT4-E express the neuronal characteristics of voltage-dependent, tetrodotoxin-sensitive, veratridine stimulated Na^+-influx and K^+-efflux and upon stimulation by cAMP, extend processes. RT4-D expresses the glial characteristics of cytoplasmic S100β protein, GFAP, P0, SCIP, and CNP and upon cAMP induction expresses high affinity GABA uptake and MBP. We are using this family of cell lines to identify the processes involved in cell fate determination. The RT4 family is well suited for using the techniques of subtractive hybridization and differential screening, because the stem cell line RT4-AC expresses many of the genes expressed in the derivative cell types. Thus, genes involved in the conversion step will be in a relatively small population. Our ultimate goal in identifying genes which are expressed differentially in the cell types is to determine which genes are involved in the cell type determination step. In addition this method should also identify neuronal genes which are differentially expressed in RT4 between the derivative and the stem cell lines and allow us to further explore the differential regulation underlying conversion.

We are beginning this project by attempting to isolate genes which are expressed only in the neuronal derivative RT4-E. After constructing an RT4-E cDNA library, we used subtractive hybridization between the library and RT4-AC RNA to create a subtracted RT4-E cDNA library. This subtracted library was screened twice with radiolabelled RT4-E cDNA and RT4-AC cDNA and clones which showed hybridization to the RT4-E probe and not the RT4-AC probe both times were selected. A preliminary screen of 3600 colonies resulted in 0.5% of the clones fulfilling our criteria. Further characterization and comparison of these clones with known genes will determine which of these clones will be tested for the ability to influence cell type conversion.

H 222 USE OF RECOMBINED EMBRYONIC STEM CELLS TO ISOLATE NEURAL STEM CELLS, Marie-Odile Ott, Sylvie Mazan, Dario Acampora and Philippe Brûlet, Unité d'Embryologie moléculaire, Département de Biologie moléculaire, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

By homologous recombination in embryonic stem cells, a reporter gene was introduced (LacZ) in several loci known to be expressed during neurogenesis in CNS, as Otx2, Otx1 and the β chain of the acethylcoline receptor. The expression of these recombined genes can be followed during development by expression of the reporter gene LacZ after introduction of such cells in recipient embryos. Otx2 is a very early marker of preneural cells during mouse embryogenesis in the headfold neuroectoderm at the presomitic stage (7.5 day gestation) and later in a region including telencephalon, diencephalon and mesencephalon.

In order to detect precursors of neural cells, "neural stem cells" the presumptive neural domains are microdissected out from chimeric embryos at different stages. Then they are put in culture conditions with different growth factors to induce expression of a neural like phenotype. The neural potencies of these cells would be characterized by expression of the reporter gene and specific markers. Transplantation of such neural precursor cells in embryos or neonates at different stages of development and/or different sites would allow to test their ability to participate to normal development.

H 223 SWISS-3T3-XYP, A CLONAL POPULATION OF MOUSE PLURIPOTENT MESENCHYMAL STEM CELLS, Shannon L. Pederson, Christina M. Klausmeyer, Steve Troum, Linda R. Adkison, John J. Rogers, and Henry E. Young, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.

Previous studies have suggested that Swiss-3T3 cells, a line of contact-inhibited fibroblastic-like cells, has the potential to differentiate into multiple phenotypes dependent on factor treatment. Recently, Troum et al. (*Clin. Res.* 41:350A, 1993) demonstrated that treatment of these cells using protocols for the isolation and cryopreservation of avian mesenchymal stem cells would elicit a cell population that demonstrated multiple phenotypes with dexamethasone treatment. As a followup to that study, Swiss-3T3 cells were processed through plating at clonal densities, growth to confluence, and cryopreservation using protocols selective for the isolation, propagation, and cloning of avian pluripotent mesenchymal stem cells. Two parameters were used to select cells for continued passage, mesenchymal (stellate) morphology and loss of contact inhibition. Cells were then plated at various densities and tested with dexamethasone, bone morphogenetic protein, muscle morphogenetic protein, and insulin to determine differentiation capabilities. Morphological, immunochemical and histochemical staining procedures were used to verify differentiated morphologies. As shown, myogenic-, adipogenic-, chondrogenic-, and osteogenic-like morphologies were apparent with these treatments. These results suggest that at least a portion of the cells within the Swiss-3T3 cell line are pluripotent stem cells.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 224 MAINTENANCE OF LONG - TERM HEMATOPOIETIC PROGENITOR CELL PROLIFERATION AND DEVELOPMENT USING A NOVEL NON - SERUM BONE MARROW CULTURE SYSTEM, Ian L.O. Ponting, Raquel Izumi and Todd Covey, Amgen, Thousand Oaks, CA 91320-1789.

The growth of hematopoietic progenitor (stem) cells is supported by bone marrow stromal cells, e.g. fibroblasts, endothelial cells, macrophages and adipocytic cells, which produce a variety of factors required by the hematopoietic progenitor cells. Many of the aspects of this process can be mimicked *in vitro* using serum supplemented "Dexter-type" long-term bone marrow cultures. However, the molecules required for this process to occur are poorly defined and the development of the progenitor cells is difficult to manipulate, problems inherent in the use of serum.

To overcome these difficulties we have developed a well defined, novel, non-serum (serum-free) culture system which uses highly purified components to maintain the long term (> 15 weeks) proliferation and development of murine myeloid progenitor cells. This process occurs in association with bone marrow stromal cells and without the addition of hematopoietic growth factors.

Microscopic examination revealed that the non-serum cultures closely resembled the serum-supplemented long-term bone marrow cultures in terms of the overall complex structure and arrangement of both the stromal and hematopoietic cells. In addition cytochemical methods showed that fibroblasts, endothelial cells (blanket cells), macrophages and adipocytic cells were all present in the non-serum cultures.

In terms of hematopoiesis a direct comparison revealed that non-serum long-term bone marrow cultures had a number of advantages over standard serum cultures. Firstly, establishment of a confluent layer of stromal cells and the initiation of hematopoiesis occurred far more rapidly and required fewer cells than for serum cultures. Furthermore, the maximum production of mature hematopoietic cells and their progenitors in non-serum media was far greater than obtained in serum - supplemented medium. The mature hematopoietic cells produced in both types of culture were primarily neutrophils and macrophages.

The greater effectiveness of the non-serum cultures in establishing and maintaining the growth of hematopoietic progenitor cells could not be explained by a beneficial effect of any individual component or group of culture system components, but instead was attributable to all of the components as a whole.

This non-serum long term bone marrow culture technique therefore has a number of advantages over prior serum supplemented cultures and will be extremely useful as a model for dissecting and understanding *in vitro* hematopoiesis and stem cell biology.

H 225 MACROPHAGE INFLAMMATORY PROTEIN-1α (MIP-1α) ENHANCES HAEMOPOIETIC PROGENITOR EXPRESSION IN SUSPENSION CULTURE, Ian B Pragnell, David J Dunlop, Edward Fitzsimons, Will Steward and Tessa J Holyoake, CRC Beatson Laboratories, Garscube Estate, Bearsden, Glasgow G61 1BD, Scotland

The rate of recovery of peripheral blood counts after high dose chemoradiotherapy and haemopoietic stem cell rescue is dependent on both the number of progenitor cells infused and the time required for these progenitors to produce mature cells. We have investigated the effect of MIP-1α (100 ng/ml) added to suspension cultures of unfractionated and highly purified murine bone marrow in the presence of Kit-ligand (KL 12ng/ml) and interleukin 11 (IL 11 60ng/ml). The addition of MIP-1α significantly enhanced the degree of amplification of both lineage committed CFU-GM and the more primitive CFU-A progenitors. Amplification factors for total progenitor numbers after 6 days incubation are as below.

Cytokines[1]	CFU-GM	CFU-A
None	4	2
KL,IL-11	150	65
KL,IL-11 plus MIP-1α	213	90

1. All cytokines provided by Genetics Institute, Cambridge, Mass.

Lethally irradiated mice transplanted with syngeneic donor cells expanded *in vitro* with the combination of KL and IL-11 showed a significant survival advantage compared with mice receiving control fresh marrow cells, and required significantly fewer cells for rescue. Similar amplifications were obtained with $Sca^{+}Lin^{-}$ progenitors. The starting populations were 95-100% blasts and 5-10% blasts were observed at the end of the incubation with two growth factors. The addition of MIP-1α resulted in an increase to 45-60% blasts. These results suggest that MIP-1α may significantly enhance blast/progenitor amplification *in vitro* in the presence of KL and IL-11.

Presenters R-Z

H 300 EMZF-1: A Zinc Finger Gene Activated at the Onset of Embryonic Hematopoiesis

Jay L. Rothstein and Barbara B. Knowles, Thomas Jefferson University, Jefferson Cancer Institute, Depart. of Micro./Immun. and Otolaryngology, 233 S. 10th St., Philadelphia, PA 19107 and The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609

Using a genetic approach, we have recently identified a novel cDNA clone from a 9.5 day blood island/yolk sac cDNA library using a human Myeloid Zinc Finger (MZF)-1 cDNA probe. This newly identified cDNA clone, Extraembryonic Mesoderm Zinc Finger-1 (EMZF-1) contains 4 C_2H_2 *Krüpple*-like zinc finger motifs which are highly homologous to MZF-1 in the zinc finger domain. Interestingly, PLZF, a newly discovered gene involved in acute promyelocytic leukemia, is also homologous to EMZF-1. Thus EMZF-1 is the third member of a newly formed hematopoietic-specific zinc finger gene family functioning to control early hematopoietic differentiation. EMZF-1 contains an alanine/arginine-rich region and a novel cysteine and histidine rich region found in several highly charged proteins involved in ion transport and transcritional regulation. At 7.5 days of mouse development EMZF-1 is expressed exclusively in the extraembryonic mesoderm of the yolk sac. The first embryonic expression observed at 9.5 days is limited to the heart followed by expression in the fetal liver by day 11. These data support recent findings of HSC activity in the heart primoidium. Expression in the adult is limited to bone marrow, heart and liver. Furthermore, EMZF-1 is regulated during the differentiation of embryonic stem cells into blood islands *in vitro*. EMZF-1 is activated in these embryoid body cultures prior to *Brachyury* and GATA-1; two genes important for mesoderm and hematopoietic differentiation respectively. We have also devised an *in vitro* culture system to study the expression of EMZF-1 in embryoid bodies, yolk sac tissue and fetal liver. Future work focusing on the potential of EMZF-1 as a molecular marker of the primordial HSCs will be explored and its function in the normal and malignant growth of hematopoietic cells elucidated.

H 301 LINEAGE ANALYSIS IN CULTURED HUMAN KERATINOCYTES,

Edward Rytina and Fiona M. Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O.Box 123, 44, Lincoln's Inn Fields, London, WC2A 3PX.

The proliferating cells in the epidermis, stem cells and transit amplifying cells, can be separated to form enriched populations of each type by their ability to adhere to extra cellular matrix (1). Stem cells adhere rapidly to type IV collagen and give rise to large colonies in culture whereas slowly adhering transit amplifying cells form small colonies of terminally differentiated cells only.

We have used a variety of lineage markers to follow individual cells and their progeny and study how different populations of cells behave when at confluence, mimicking the epidermis in a steady state when the rate of cell renewal is balanced by that of differentiation.

We have evidence that clones derived from the putative stem cell population are large and long lived compared to clones founded by the transit amplifying population which are rapidly lost from reconstituted epidermis.

By using various culture techniques we can look at how the proliferating and differentiating cells of the epidermis may be organised *in vivo* and whether there is a relationship between the dermis and the behaviour of the different types of epidermal cells in the steady state.

1. Jones P. H. and Watt F. M., Cell (1993): 73, 713-724.

H 302 *Abstract Withdrawn*

H 303 SERUM FREE CULTURE OF PURIFIED $CD34^+$ CELLS YIELDS LARGE EXPANSIONS OF HEMATOPOIETIC PROGENITORS.

Lisa Schain, Mark Harvey, Michelle Wysocki, William Biddle, Annemarie Moseley, Thomas B. Okarma and Jane Lebkowski, Applied Immune Sciences, Inc., Santa Clara, CA; Life Technologies, Inc., Grand Island, NY.

Cultured human bone marrow or peripheral blood progenitors could serve as transfusion support to minimize neutropenic periods post high dose or dose intensification chemotherapy for a variety of malignancies. Towards this end we explored the use of serum free media (Gibco, 16AB) for the expansion of purified $CD34^+$ cells from bone marrow and peripheral blood. For these studies, $CD34^+$ cells were captured on AIS MicroCELLector® CD34 and clinical scale CELLector™ CD34 devices and cultured directly in the device at 37°C in 5% CO_2 with IL-1, IL-3 and stem cell factor. Half media changes were made twice weekly during the approximately 4-5 weeks of culture. On average, cell expansions were 15-25 fold and 50-100 fold through the first and second weeks of culture respectively, and were 5 fold higher than those in identical cultures containing fetal calf serum based media. Cell numbers plateaued after 3-4 weeks in culture. Cultures maintained in serum free conditions produced 7-50 fold expansions of CFU-GM and 10-300 fold increases in BFU-E which peaked at 7-14 days of culture. In contrast, only 3-10 fold expansions of CFU-GM and no proliferation of BFU-E were observed with identical cultures containing fetal calf serum. Moreover, the expansion of $CD34^+$ cells was enhanced with the use of serum free media, with up to 16 fold increases in $CD34^+$ cells after 7-10 days in culture. The rate of expansion and differentiation of the $CD34^+$ cells could be controlled by the appropriate combination of growth factors used during culture. Similar results were observed for the expansion of $CD34^+$ cells from G-CSF mobilized peripheral blood. Additionally, similar results were observed when clinical scale expansions were performed. The characterization of primitive stem cell expansion and platelet progenitors in this system is currently ongoing. These data establish the feasibility of using this system for large scale clinical expansions of $CD34^+$ cells and demonstrate the superior performance of the serum free culture conditions for the maintenance and expansion of hematopoietic progenitors.

H 304 ANALYSIS OF MULTIGENE FAMILIES BY DNA FINGERPRINTING OF CONSERVED DOMAINS: EXPRESSION PATTERNS OF PROTEIN TYROSINE PHOSPHATASES IN HEMATOPOIETIC CELLS, Gunter Schumann and Thomas Boehm, Molecular Medicine Group, Department of Internal Medicine I (Hematology/ Oncology), University of Freiburg, Germany.

We have developed a new procedure based on DNA fingerprinting of conserved domains to detect the expression of individual members of multigene families. After mRNA extraction and cDNA synthesis, the amplification is done with asymmetrically labeled primers specific for a conserved domain of a given multigene family. The PCR products are digested with a panel of restriction enzymes and run on a sequencing gel for autoradiography. The resulting band patterns represent a diagnostic fingerprint of the expression pattern of individual family members.

With this approach at least 20 different mouse protein tyrosine phosphatases (PTP) during development could be detected (Ref.). The majority of these PTP show developmentally regulated expression patterns, some displaying a unique tissue specificity. Here, we show that several human α/β- and γ/δ- T-cell lines display a differential expression pattern of PTP as detected by distinct diagnostic fragments in the fingerprint analysis.

We are now applying this method to investigate the expression pattern of protein tyrosine phosphatases in various sub-fractions of CD34-positive human hematopoietic progenitor cells.

Reference: Boehm, T., Analysis of multigene families by DNA fingerprinting of conserved domains: directed cloning of tissue-specific protein tyrosine phosphatases. Oncogene (1993), 8, 1385-1390

H 305 ISOLATION OF MARKERS OF EARLY MESODERM IN AN *IN VITRO* EMBRYONIC STEM CELL DIFFERENTIATION SYSTEM, Michael M. Shen and Philip Leder, Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115

We have been interested in understanding how embryonic stem (ES) cells commit to alternative lineages during their differentiation. To pursue this issue, we have developed an approach for isolating embryonic genes that are markers of primitive ectoderm and early mesoderm during ES embryoid body differentiation *in vitro*. Our previous experiments have shown that genes expressed in untreated embryoid bodies versus ones treated with Leukemia Inhibitory Factor (LIF) will include markers for these cell types (M.M. Shen and P. Leder, *PNAS* 89, 8240-8244 (1992)).

In order to identify such genes, we have utilized the differential display technique, a PCR-based protocol that is capable of detecting rare differences between two RNA populations. This technique has shown that very few differences in gene expression exist between untreated and LIF-treated embryoid bodies at days 4 and 6 of *in vitro* culture, prior to the appearance of markers of terminal differentiation. We have successfully isolated several novel genes that are differentially expressed between these two populations. Whole-mount *in situ* hybridization analysis of one of these genes shows that it has specific expression patterns during early mouse embryogenesis, with expression in forelimb buds, somites, and the first branchial arch of day 9.5 embryos. We will present our current results on the characterization of these differentially expressed genes.

H 306 TARGETED MUTAGENESIS OF THE STEM CELL SPECIFIC TRANSCRIPTION FACTOR OCT-4, Austin G. Smith, Branko Zevnik, Peter Mountford, Annette Duwel, Jennifer Nichols and Meng Li, AFRC Centre for Genome Research, West Mains Rd, Edinburgh, EH9 3JQ, UK

The pluripotent nature of early embryo cells is likely to be governed by specific gene transcription. A candidate transcription factor is the POU-domain protein Oct-4 (also known as Oct-3). Expression of Oct-4 is confined to pluripotent embryo cells *in vivo* and to undifferentiated embryonic stem (ES) cells in culture where it constitutes the major octamer-binding protein. In order to assess the function of Oct-4, we have used homologous recombination in ES cells to disrupt the Oct-4 gene.

Isogenic DNA was employed to construct a promoterless targeting vector in which the DNA-binding domain was replaced by a *lacZ-neo* (βgeo) fusion gene containing its own polyadenylation site. In the targeting event, the βgeo fusion gene is spliced to exon one of the Oct-4 gene via a splice acceptor sequence situated 5' of the βgeo gene. Functional βgeo fusion protein is produced independent of the Oct-4 reading frame by employing the EMC virus internal ribosomal entry site (IRES). The integrated lacZ reporter enables visualisation at the cellular level of Oct-4 expression sites, whilst the deletion of the entire POU-domain will allow definitive determination of the requirements for Oct-4 in embryogenesis. Electroporation of the targeting vector into ES cells resulted in a high frequency (>80%) of targeted clones, as determined by Southern hybridisation using 5' and 3' flanking probes. RNA analysis reveals the presence of the predicted Oct-4-IRES-βgeo fusion transcript in Oct-$4^{+/-}$ ES cells. Expression of lacZ is confined to undifferentiated cells *in vitro* and to pluripotent early embryo cells *in vivo*, reflecting the normal pattern of Oct-4 expression. Chimaeras have been generated which give rise to ES cell derived progeny.

H 307 TEMPORAL RELATIONSHIP BETWEEN HEMATOPOIETIC PROGENITOR CELL DEVELOPMENT AND CD34 EXPRESSION DURING MURINE EMBRYONIC STEM CELL DIFFERENTIATION, Orla M. Smith, Mary Jo Fackler, Diane S. Krause, Michael I. Collector, Saul J. Sharkis and W. Stratford May, Johns Hopkins Oncology Center, Baltimore, MD 21231

Mouse embryonic stem (ES) cells provide a unique system with which to examine early events in mammalian hematopoiesis. When cultured in vitro in the presence of leukemia inhibitory factor (LIF) ES cells maintain their undifferentiated, totipotent phenotype. However, on removal of LIF, ES cells spontaneously differentiate forming three-dimensional structures termed embryoid bodies (EB) which are comprised of progenitor cells of different lineages. CD34 is recognized as the only developmental stage-specific, surface antigen expressed on all lineages of hematopoietic progenitor cells (HPCs). We have used differentiating ES cells to examine the potential role of CD34 in the earliest events of hematopoietic development. CD34 expression in differentiating EBs was determined by Northern and RT PCR methods. Analysis of CD34 expression showed very low levels of mRNA in undifferentiated ES cells and up regulation of mRNA as HPC development occurred. Embryoid bodies at day 1 - 6 of differentiation were dissociated, 10^5 single cells were plated in growth factor supplemented semisolid medium, and CFU were enumerated 14 days later. No CFU were seen in cultures of day 1 EB cells whereas a small number of CFU were observed in cultures of day 2 EB cells. As EB differentiation progressed the number of cells competent to form HPCs, including BFUe, CFU GEMM and CFU GM, increased. Our data suggest that ES cells become hematopoietically competent between 24 - 48 hours following initiation of differentiation.

Day of Differentiation of Embryoid Bodies	CFU/10^5 cells BFUe	CFU GEMM	CFU GM
day 1	0 ± 0	0 ± 0	0 ± 0
day 2	0 ± 0	0 ± 0	11.4 ± 5.8
day 3	0.5 ± 1.0	2.5 ± 3.0	8.5 ± 4.4
day 4	5.8 ± 4.4	31.5 ± 5.3	66.7 ± 3.3
day 5	6.8 ± 4.3	33.7 ± 4.9	62.5 ± 10.1
day 6	25.7 ± 5.3	13.6 ± 4.6	113.7 ± 6.4

The association between CD34 expression and HPC development is consistent with an important role for CD34 during the early stages of hematopoiesis.

H 308 HUMAN HEMATOPOIETIC STEM CELLS CULTURED *IN VITRO* ENGRAFT SCID MICE ONLY AFTER INTRAPERITONEAL INJECTION. S. Spence, W. Murphy, F. Ruscetti, J. Gooya, S. Funakoshi, D. Longo and J. Keller, BCDP-PRI/DynCorp, LLB-BRMP, NCI-FCRDC, Frederick, MD 21702.

Engraftment of SCID mice with human hematopoietic cells provides a unique model to study the efficiency of retroviral gene transfer and expression in primitive stem cells. Most protocols for gene transfer into human hematopoietic cells require cycling of cells in liquid culture in the presence of human IL-3 and stem cell factor (SCF) for 2 to 6 days before transfer to the recipient. Therefore we have evaluated engraftment of SCID mice by human bone marrow and cord blood hematopoietic cells maintained under these conditions for six days. SCID mice received 0 to 200 rads of irradiation and anti-asialo GM-1 before transfer of 2 to $3x10^7$ human cells by intravenous (IV) or intraperitoneal (IP) routes. Recipients then received human SCF and an IL-3/GM-CSF fusion protein (PIXY321) every other day. Engraftment was evaluated after at least four weeks by the ability of bone marrow and spleen cells harvested from recipients to form colonies in soft agar under conditions specific for the growth of committed human progenitors. Colonies were then evaluated by PCR to confirm human origin. Both human bone marrow and cord blood hematopoietic cells maintained in culture before transfer to SCID mice engrafted only after IP injection, and not when injected IV. Cord blood cells injected IP engrafted the bone marrow in 9 of 17 recipients and the spleen in 3 of 17 recipients. Spleen engraftment was never seen in the absence of bone marrow engraftment. In comparison, bone marrow cells injected IP engrafted the marrow in 7 of 7 mice and the spleen in 4 of 7 mice. Interestingly, engraftment of cord blood hematopoietic cells was observed when SCID mice were not irradiated, suggesting that competition for space in the marrow is not a limiting factor in the SCID-human model. Thus, human hematopoietic cells cultured under conditions optimal for retroviral gene transfer are capable of engraftment in SCID mice.

H 309 LY-6 EXPRESSION DURING EMBRYONIC STEM CELL DERIVED PRECUSOR DEVELOPMENT. William L. Stanford and H. Ralph Snodgrass. Dept. of Microbiology and Immunology, Univ. of North Carolina, Chapel Hill, NC and Progenitor, Inc., Columbus, OH.

The in vitro differentiation of murine Embryonic Stem (ES) cells mimics the differentiation of non-hematopoietic stem cells into hematopoietic stem cells during early embryonic development. ES cell derived cystic embryoid bodies resemble yolk sac development both morphologically and by gene expression. Although yolk sac derived hematopoietic stem cells can give long-term repopulation to lethally irradiated animals, long-term reconstitution using in vitro differentiated ES cells has been largely unsuccessful. To better define hematopoietic development during ES cell differentiation, we are studying the expression of the Ly-6 gene family in colony-forming precursors. The Ly-6 gene family is a large family of glycosyl phosphatidyl inositol linked cell surface proteins expressed in hematopoietic and non-hematopoietic tissues. The expression of Ly-6A (Sca-1) on hematopoietic progenitors has been exploited by many laboratories to enrich for progenitors from the fetal liver and adult bone marrow. The Ly-$6A^+$Thy-1^{lo}Lin$^-$ cell population is heterogenous, containing cells capable of long-term reconstitution as well as more mature day 10 spleen colony forming units. Ly-6B, Ly-6C, Ly-6G, and ThB are additional Ly-6 proteins expressed in the bone marrow and peripheral lymphoid organs. We will present RT-PCR and FACS analyses used to determined the expression of these proteins during the development of in vitro ES cell derived colony forming precursors and compare their expression to Ly-6 expression on fetal liver and adult bone marrow derived hematopoietic precursors.

H 310 **BOVINE FETAL DEVELOPMENT OCCURS WHEN BOVINE EMBRYONIC STEM (ES) CELLS ARE USED IN NUCLEAR TRANSFER PROCEDURES,** **Steven L. Stice and Nick S. Strelchenko, American Breeders Service, Deforest WI 53532**

The production of ES cells in domestic animals has remained an elusive goal for the last several years. We describe here a method of producing bovine ES cells from blastocyst stage embryos. Both the totipotency and the pluripotency of the ES cell lines were tested in vitro and in vivo. Bovine blastocysts were disaggregated and seeded on top of a feeder layer which consisted of mitomycin treated mouse primary embryonal fibroblasts. ES cells were established in MEM-Alpha medium supplemented with 10% fetal calf serum and 0.1 mM 2-mercaptoethanol. ES cell passages were started approximately 7 to 10 days later. ES cell colonies consisted of a monolayer of cells with distinct nuclei. These nuclei occupied most of the ES cells' volume. After mechanical disaggregation, ES cells were passaged onto a new feeder layer. ES cells have been maintained in this manner for over an 11 month period. The ES cells can also spontaneously differentiate into embryonal bodies and endoderm like cells. Micrographs of ES cells induced to differentiate will be presented. The ES cells were also used as nuclear donors in nuclear transfer procedures. Following the enucleation of in vitro matured metaphase II bovine oocytes, a single ES cell was transferred into the perivitelline space of each enucleated oocyte using micromanipulation techniques. Electrofusion was used to fuse the membranes between the oocyte cytoplasm and the ES cell. Nuclear transfer embryos were then artificially activated. In vitro development to blastocyst stage was obtained and embryos were transferred into recipient females. Some pregnancies reached 55 days before fetal heartbeats were lost. Recovered fetuses showed no gross abnormalities. Pregnancies could be extended further if chimeras were formed by combining fertilized embryos with nuclear transfer embryos. ES cell contribution to three different organs has been confirmed in an 85 day chimeric fetus. To date, there are five ongoing putative chimeric pregnancies which are in the third trimester. Although totipotency of bovine ES cells has not yet been established, their contribution to different fetal organs has been verified.

H 311 GENERATION OF $CD1^+$ DENDRITIC CELLS FROM PERIPHERAL BLOOD STEM CELLS OF NORMAL HEALTHY ADULTS

Dirk Strunk, Adelheid Elbe and Georg Stingl, Department of Dermatology, DIAID and Vienna International Research Cooperation Center, University of Vienna Medical School, Vienna, Austria

Dendritic cells (DC) represent a family of bone marrow-derived MHC class II-bearing leukocytes. They reside in small numbers in both lymphoid and non lymphoid tissues and possess a unique stimulatory capacity for primary immune responses. So far, limitations in DC numbers as well as well as tedious isolation procedures have hampered the progress in this field. Recently, several investigators have successfully generated immunostimulatory cells with features of DC/Langerhans cells (LC) from bone marrow and from cord blood samples, in vitro. In an attempt to find a more practical source of realistic numbers of human DC, we utilised peripheral blood from normal healthy adults.

For this purpose, PBMC from healthy volunteers were isolated over density gradients and depleted of mature T-cells and monocytes. $CD34^+$ hematopoietic progenitor cells (HPC) were further enriched by means of anti-CD34-reactive paramagnetic beads to a purity of consistently >95%. Most peripheral blood HPC from normal healthy adults express MHC class II-molecules but do not react with lineage specific antibodies.

After a two week in vitro stimulation of these cells with various cytokine combinations, we found that the presence of GM-CSF and TNF resulted in the emergence of $CD1a^+$/HLA-DR^+/$CD11b^+$/$CD4^+$/$CDw32^{+/-}$/$CD40^{+/-}$/$CD3^-$/$CD19^-$ cells, as detected by single and dual colour immunostaining techniques. The phenotype of these cells is compatible with that of the LC lineage. Their T-cell stimulating capacity, as investigated in a 1° allogeneic mixed leukocyte reaction, far exceeds that of mononuclear phagocytes.

These in vitro propagated, peripheral blood HPC-derived $CD1a^+$ cells could be valuable tools for vaccination purposes.

H 312 ADHESION OF RAT MYELOID PRECURSORS TO MARROW STROMAL CELLS: INHIBITION BY AN ANTIBODY TO THE ST3 ANTIGEN. A.K. Sullivan and Jing An, McGill University Hematology Program - Royal Victoria Hospital Unit, Montreal, Canada.

Introduction: Previous experiments showed that a murine monoclonal antibody called "ST3" recognized >95% of fibroblastoid cells (FB) grown from rat bone marrow, but a minority of cells from various non-hemopoietic tissues (Lab Invest. 60:667, 1989). Ab "ST4" recognized most "non-hemopoietic" fibroblasts, but few marrow FB. An immunohistologic survey of other organs revealed that the ST3 antigen was expressed also in the thymic cortex (lymphocytes and nurse cells) and on a subpopulation of neurons. **Purpose**: To test the possible role of the ST3 antigen in adhesion of committed myeloid precursors to $ST3^+$ fibroblasts. **Methods**: Rat bone marrow cells ($1.5x10^5$) were incubated for 3 hr on a preformed layer of $ST3^+$ marrow-derived fibroblastoid cells, after which CFU-C and CFU-e were measured in the adherent and non-adherent fractions. The effect of pre-incubation of the stromal cells with antibody also was tested **Results**:

Stromal layer	CFU-C (non-adh)	CFU-C (adh)	CFU-E (non-adh)
none	108±1.5	<1±0.2	125±1.7
FB+PBS	57±2.4	31±0.6	102±2.8
FB+Neg Ab	60±2.7	28±0.8	100±2.2
FB+ST3 Ab	93±1.5	16±1.2	108±1.0
FB+ST4 Ab	59±1.8	30±1.1	112±2.6

CFU-C, but not CFU-e, were partially removed by the stromal layer; the adhesion was blocked by anti-ST3, but not control antibodies. Also, similar inhibition was seen after pre-incubating the stromal layer but not marrow cells with the antibody. **Conclusion:** CFU-M, but not CFU-e, adhered to marrow fibroblastoid cells through a process partially blocked by the ST3 antibody. This may be caused by direct interference with the adhesion molecules, or indirectly through other accessory structures on the cell surface.

H 313 CONTINUOUS CULTURES OF PLURIPOTENT FETAL HEPATOCYTES DERIVED FROM THE 8-DAY EPIBLAST OF THE PIG, Neil C. Talbot, Caird E. Rexroad, Jr., Anne M. Powell, Vernon G. Pursel, Thomas J. Caperna, Sherry L. Ogg and Neil D. Nel, U.S. Dept. of Agriculture, Agricultural Research Service, Beltsville Agric. Res. Ctr., Beltsville, MD 20705

Continuous cultures of pluripotent parenchymal hepatocytes were derived from the epiblasts of 8-day-old pig blastocysts. The cells were polygonal and had phase-contrast dark, granular cytoplasm with prominent nuclei and nucleoli. These feeder-dependent cell cultures differentiated into large secretory duct-like structures or formed small canaliculi. Alternatively, the cells accumulated droplets that stained intensely with Oil Red O, a lipid-specific stain. Alpha-fetoprotein (AFP) and albumin mRNA expression increased as the cells differentiated in culture. Serum-free medium that was conditioned by the cells contained transferrin and albumin. The growth and viability of the cells were inhibited by transforming growth factor ß1 (TGFß1) at concentrations ≥1 ng/ml. The cell cultures grew slowly with doubling times of 3 to 4 days. One of the cultures, pig inner cell mass-19 (PICM-19), was passaged continuously for over 2 years [>100 population doublings (PD)] and appears to be an infinitely self-renewing cell population. The stem cell characteristics of the epiblast-derived fetal hepatocytes indicate that the cells may be unique for investigations of liver differentiation and organogenesis.

H 314 CLONAL ANALYSIS OF EMBRYONIC CEREBRAL CORTICAL PROGENITOR CELLS REVEALS A RARE TOTIPOTENT STEM CELL, Sally Temple and Andrew Davis, Department of Pharmacology and Division of Neurosurgery, Albany Medical College, Albany NY 12208.

Fundamental to understanding the role of lineage in neural development is the determination of progenitor diversity within CNS proliferative zones. In an attempt to directly assess this, we have taken cortical ventricular zone cells from E12 and E14 rats and cultured them as single cells in separate culture wells. Cells exhibited different fates under identical culture conditions, suggesting that they are intrinsically heterogeneous. The majority, 80-90%, generated small clones of 2-12 neurons. Only 2% of the clones consisted solely of glial cells; these clones were of 4-40 cells. 10% of the cells generated small clones (av. size 25 cells) of neurons and glia. In contrast, approximately 7% of the cortical cells divided rapidly, generating hundreds of neuronal and glial progeny. Because of their huge proliferative capacity and their multipotential fate we have called these cells "stem cells". Around 40% of the cortical stem cells at E12-E14 are totipotent, generating numerous neurons, astrocytes and oligodendrocytes. These results suggest that while a majority of cells in the cortical ventricular zone are restricted in their proliferative capacity and cell type, an as yet uncharacterized population of pluripotential stem cells are also present. (aided by the Klingenstein Fellowship Award in the Neurosciences and by the Basil O'Connor Starter Scholar Research Award No. 5-FY91-0636 from the March of Dimes Birth Defects Foundation and by the Henry Schaffer Fellowship from AMC)

H 315 LEUKEMIA INHIBITORY FACTOR PREVENTS BONE MORPHOGENETIC PROTEIN-INDUCED CHONDROGENESIS IN MOUSE MESENCHYMAL STEM CELLS, Sandi A. Tincher, Michelle L. Reeves, Kathi H. Davis, John J. Rogers, and Henry E. Young, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.

Leukemia inhibitory factor (LIF) is a cytokine with a broad range of effects on diverse cells types. Of particular interest is its reported suppression of spontaneous differentiation by totipotent stem cells derived from the inner cell mass of mouse blastocysts. These studies suggested a potential for LIF to effect mesenchymal stem cell differentiation during tissue restoration. The purpose of this study was to determine the ability of LIF to effect proliferation, progression, and/or induced commitment of cultured mouse pluripotent mesenchymal stem cells. Bone morphogenetic protein (BMP) was used to induce chondrogenic commitment and differentiation, while insulin was used to stimulate progression. Swiss-3T3-XYP mesenchymal stem cells were plated at various densities, treated with 250 to 4000U LIF/ml with and without 50 to 200 ng/ml rh-BMP with and without 2µg/ml insulin, and assayed for cellular proliferation, progression, and/or chondrogenic commitment. Immunochemical and histochemical stains were used to verify adult differentiated phenotypes. The results show that LIF effectively inhibited chondrogenic progression and differentiation, while having no apparent effect on cellular proliferation. The results suggest that LIF may be useful in conjunction with non-fibrogenic differentiation factors to prevent premature differentiation during bio-active factor-induced tissue restoration.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 316 Heterogeneity of mouse hematopoietic stem cells separated by counter-flow centrifugal elutriation

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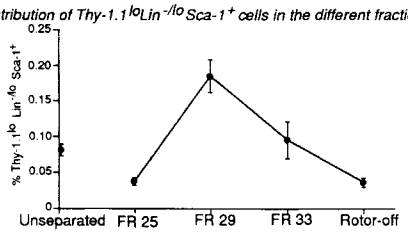
Mouse hematopoietic stem cells (HSCs) are highly enriched in the rare (~0.05 %) Thy-1.1^{lo} Lin$^{-/lo}$ Sca-1^{+} bone marrow (BM) cells. Using counterflow centrifugal elutriation (CCE), Jones et al separated mouse BM into four fractions, which were subsequently placed into cellular assays to test various hematopoietic functions (Jones et al.Nature 347:118, 1990). Of the four fractions tested, FR 25 (mostly small cells) appeared to be deleted or devoid of CFU-GM and CFU-S as well as radioprotective capacity, but played a dominant role in multilineage engraftment (LTMR). A second fraction, called rotor-off (RO) (mainly blast cells) was reported to be highly enriched for CFU-GM, CFU-S, and radioprotective capacity, but devoid of LTMR. They concluded that there are two vital classes of engrafting cells: committed progenitors, which provide initial, unsustaining engraftment, and HSC, which produced delayed, but durable engraftment. We repeated the CCE technology of Jones et al. to determine the distribution of Thy-1.1^{lo} Lin$^{-/lo}$ Sca-1^{+} cells in each fraction, and qualitatively whether it was true that committed progenitors capable of radioprotection and various colony assays could be separated from true HSCs. Here we report that Thy-1.1^{lo} Lin$^{-/lo}$ Sca-1^{+} cells in the fractions described (FR 25, 29, 33 and RO) all contained highly enriched populations of cells capable of LTMR, CFU-S, and radioprotection capacity. We conclude that this technology does not separate committed progenitors from HSC, and the results reported by Jones et al. reflect the distribution of Thy-1.1^{lo} Lin$^{-/lo}$ Sca-1^{+} HSCs in CCE fractions.



H 317 MARKERS FOR UNDIFFERENTIATED COLON CARCINOMA CELLS IDENTIFIED BY SUBTRACTION HYBRIDIZATION AND ARBITRARILY PRIMED PCR,

Nico van Belzen, Michaël P.G. Diesveld, Angelique C.J. van der Made, Remko Vlietstra, Jan Trapman and Fred T. Bosman, Department of Pathology, Erasmus University, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands

In general, undifferentiated (U) colon tumors are more agressive than differentiated (D) tumors are. The aim of this study is, to identify and characterize markers for the undifferentiated state of normal and neoplastic colon epithelium. As a model system, we investigate the colon carcinoma cell line HT29-D4 (Dr. J. Fantini) that can be induced to differentiate *in vitro*. cDNA libraries from both U and D cells are used as starting material for PCR-based subtraction hybridization. In addition to subtraction by biotinylation, we developed a digestion-based subtraction procedure. Furthermore, mRNA from U and D cells is 'fingerprinted' by arbitrarily primed PCR on first strand cDNA. The mRNAs encoding hsc70, adenylosuccinate lyase, α-tubulin, and two unknown transcripts, were found to be more highly expressed in U as compared to D cells. The expression of these transcripts in colorectal neoplasms will be investigated.

H 318 CHARACTERIZATION AND PURIFICATION OF OSTEOGENIC STEM CELLS FROM MURINE BONE MARROW BY TWO COLOR CELL SORTING USING ANTI-SCA-1 MONOCLONAL ANTIBODY AND WHEAT GERM AGGLUTININ.

Van Vlasselaer, P., Activated Cell Therapy, Inc. 219 Norht Bernardo Avenue Mountain View, California 94043

Osteogenic cells were sorted from bone marrow of 5-Fluorouracil (5-FU) treated mice based on light scatter characteristics, Sca-1 expression and their binding to wheat germ agglutinin (WGA). Four sort gates were established using forward (FSC) and perpendicular (SSC) light scatter and were denominated: FSC^{high} SSC^{low},FSC^{low} SSC^{high}, FSC^{low} SSC^{low} and FSC^{high} SSC^{high}. Sorted cells from the FSC^{high} SSC^{high} gate but not from the other gates synthesized alkaline phosphatase (ALP), collagen and ostocalcin and formed a mineralized matrix in culture. Similar results were obtained with marrow depleted from T, B and NK cells, granulocytes, macrophages and erythrocytes. Approximately 95% of the FSC^{high} SSC^{high} cell population of this "lineage negative" (Lin^{-}) marrow expressed the Sca-1 antigen (Sca-1^{+}) and bound WGA. Three additional sort windows were established based on WGA binding intensity and were denominated" Sca-1^{+} WGA^{dull}, Sca-1^{+} WGA^{medium}, Sca-1^{+} WGA^{bright}. Sorted cells from the Sca-1^{+} WGA^{bright} gate but not from the other gates synthesized bone proteins and formed a mineralized matrix. However, they lost this capacity upon subcultivation. Further immunophenotypic characterization reveiled that FSC^{high} SSC^{high} Lin^{-}Sca-1^{+}WGA^{bright} cells expressed stromal (KM16) and endothelial (Sab-1 and Sab-2) markers but not hematopoietic surface markers such as c-kit and Thy1.2.

H 319 MURINE KERATINOCYTE CULTURE AND THE INFLUENCE OF FIBROBLAST FEEDER LINES.

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In vitro culture of mouse keratinocytes have only a limited passage life. Even neonatal keratinocytes when grown at clonal density can only be passaged once or twice. We have investigated the role of connective tissue factors in the maintenance of mouse keratinocytes with a proliferative phenotype *in vitro* by analysing the efficiency of various fibroblast cell lines to act as feeder support in clonal culture systems. Striking differences have been observed in colony morphology and passage life on different feeder layers, and the factors involved in this phenomenon and its' implications for the maintenance of primitive cells in culture are discussed.

H 320 EXPRESSION OF FLK2 / FLT3 RECEPTOR TYROSINE KINASE IN TRANSGENIC MICE, Cornelius F. Waller*, Madeline M. Fort#, Mercedes Dosil*, Drew M. Pardoll# and Ihor R. Lemischka*, *Department of Molecular Biology, Princeton University, Princeton NJ 08544 and #Department of Oncology, Johns Hopkins University, Baltimore MD 21205.

Signal transduction initiated by the interactions of growth factors with their specific receptors is an important mechanism of regulating normal cell growth and differentiation. Flk2/Flt3 is a recently identified receptor tyrosine kinase expressed in primitive hematopoietic cells. In order to elucidate the role of Flk2/Flt3 in hematopoiesis, we developed several constructs containing the cDNA of wild type or mutated murine Flk2/Flt3 driven by different tissue specific promoters. Furthermore, to circumvent the cognate ligand requirement for Flk2/Flt3 we constructed a chimeric receptor consisting of the extracellular ligand-binding domain of the human colony-stimulating factor 1 (CSF-1) receptor (cfms) with the transmembrane and tyrosine kinase domains of murine Flk2/Flt3. Previous in vitro analyses have shown biological activity of the chimeric receptor upon stimulation with human CSF-1. The constructs were used to generate transgenic mice. Hematopoietic tissues of animals carrying the chimeric transgene were treated in vitro with human CSF-1 and effects on hematopoietic cell development and differentiation were analysed.

H 321Phenotypic and Functional Heterogeneity of Fetal Bone Marrow Mesenchymal Stem Cells. Edmund K. Waller, Fridtjof Lund-Johansen, Johanna Olweus, Shiang Huang, Leon WMM Terstappen. Stem Cell Biology Program, Becton Dickinson Immunocytometry Systems, San Jose, CA 95131-1807

A variety of pluripotent progenitor cell types exist within fetal bone marrow cell preparations. We have recently described a novel, CD34+38-HLADR- stem cell population that possesses the ability to differentiate along a stromal lineage, and that creates an in vitro microenvironment that supports the growth of hematopoietic cells. The stromal and hematopoietic stem cell populations within fetal bone marrow were studied by single cell sorting of phenotypically defined cell populations into a variety of growth media. The mean cloning efficiency of singly sorted CD34+38-DR- stromal cell progenitors in media containing 25% serum, IGF-1, and bFGF, was 1.4% (+/- S.D. of 1.9% in 9 experiments). These cultured cells grew initially as an adherent monolayer that later developed into a three-dimensional stromal structure consisting of chords of fibrous material suspended above the bottom of the culture plate. In addition, the CD34+38-DR- population contained a separate population of hematopoietic stem cells (cloning efficiency of 10%) which formed hematopoietic colonies in growth media containing a cocktail of hematopoietic growth factors. Further sub-dividing the CD34+38-HLADR- population using monoclonal antibodies to CD30 and CD50 has increased the cloning efficiency of singly sorted stromal progenitors to over 5%. Using Thy-1 and CD49b revealed further heterogeneity within the CD38- HLADR- fraction of bone marrow. The CD34+Thy- cells failed to grow in culture; in contrast, the CD34-Thy+ cells had a cloning efficiency of over 20% when sorted as single cells. The growth properties of these CD34- bone marrow cells were markedly different from that of the CD34+CD38-HLADR- cell: the CD34-Thy+ and CD34-CD49b+ cells grew more rapidly; had a higher cloning efficiency upon replating and grew exclusively as adherent stromal cells, and never as cells of the hematopoietic lineages. These data indicate the existence of three separate stem cell compartments within fetal bone marrow: hematopoietic stem cell and stromal stem cell populations, defined by their expression or lack of expression of CD34, and a sub-population of CD34+ cells that includes both hematopoietic and stromal cell progenitors.

H 322 IDENTIFICATION OF E2A-LIKE PROTEIN INVOLVED IN THE REGULATION OF RAT COLLAGEN II GENE EXPRESSION DURING CHONDROCYTE DIFFERENTIATION, Liqun Wang, Richard Balakir, Patricia Precht and Walter E. Horton, Jr., Laboratory of Biological Chemistry, Gerontology Research Center, NIA / NIH, 4940 Eastern Ave., Baltimore, MD 21224

We have previously identified an enhancer sequence, (5'-CACAATGC-3'), located in the first intron of the Collagen II gene, that binds chondrocyte specific complexes. Nuclear extracts prepared from both *in vivo* and *in vitro* differentiating cells showed correlation between binding activity and the extent of differentiation. Since this sequence resembles an E-box sequence, we have tested the possibility that it could serve as a binding site for HLH proteins. First, bacterially-expressed Myo D and E47 were able to bind to this sequence as homodimers or heterodimers. The dimers competed with chondrocyte-specific protein complexes for binding to the same sequence. Mutations altering the second C, the T or the G all resulted in loss of the binding activity. Secondary, an E2A antibody was able to disrupt the binding of chondrocyte specific complexes to the DNA, suggesting the presence of E2A-like protein in the binding complex. Immunopercipitation of labeled chondrocyte extract with the antibody showed increased amount of E2A protein from undifferentiated limb buds to differentiated chondrocyte. Finally, an E47 antisense construct effectively suppressed the enhancer activity when co-transfected with a reporter construct containing the Collagen II promoter / enhancer in differentiated chondrocytes. All the evidence suggested that the Chondrocyte marker, Collagen II, is likely regulated by HLH type proteins during differentiation as seen in many other cell lineages.

H 323 A Dominant Negative Form of SCIP (Suppressed cAMP Inducible *POU*) in Transgenic Mice Results in an Embryonic Lethal Phenotype. David E. Weinstein and Greg Lemke, Laboratory of Molecular Neurobiology, The Salk Institute, La Jolla, CA. 92037.

SCIP is expressed predominantly in Schwann cells as they undergo a proliferative burst just prior to myelination. *In vitro* evidence from our lab has demonstrated that SCIP acts to repress the myelin structural genes Protein 0(P0) and myelin basic protein. We have made a construct of SCIP encoding a protein which is missing the amino terminal 150 amino acids. Gel shift assays demonstrate that this truncated protein binds cognate DNA, while *in vitro* transcription assays demonstrate that it fails to act as a regulator of transcription. The truncated SCIP construct is under the transcriptional regulation of the P0 promoter, which is active both in myelinating Schwann cells, and embryonically in an as yet undefined cell population. We have generated two lines of mice which harbor the transgene, and in F2 animals a number of the embryos demonstrate gross developmental deformities in tissues outside of the central nervous system. We are currently generating additional lines of mice to investigate whether this phenotype is an insertional event or the result of precocious expression of our transgene as a result of early P0 expression. Interestingly, individuals from both lines of mice which survive to adulthood show very similar phenotypes, which are consistent with peripheral neuropathy. Histologically, the proximal sciatic nerves of these adult animals are often bifurcated, demonstrate abnormal fasciculation and appear to have an abnormally large number of large caliber axons as opposed to small axons. The differences seen in the embryonic and adult phenotypes might reflect the temporal differences in expression of the transgene and or the differences between homo and heterozygotes.

H 324 JAK2 TYROSINE KINASE INDUCTION BY EPO AND GM-CSF: FUNCTION AND STRUCTURE ANALYSIS UTILIZING MUTATED CONSTRUCTS

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The Janus family of kinases have been shown to be activated in response to a number of cytokines that influence stem cell mitogenesis and differentiation. The Janus family of kinases are characterized by the presence of twin kinase domains, as well as other highly conserved regions. We demonstrate that the Epo and GM-CSF receptor membrane proximal region is necessary for Jak kinase activity in response to ligand. To begin assessing the function of the Jak kinase domains, mutations consisting of deletion and chimerics utilizing two family members were constructed. The relationship of the domains with their function and the association with various receptors will be assessed in this manner and reported.

H 325 EFFECTS OF PLATELET-DERIVED GROWTH FACTORS ON MOUSE MESENCHYMAL STEM CELLS, Robert P. Wright, John J. Rogers, and Henry E. Young, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.

Platelet-derived growth factors (PDGF) have been used experimentally to accelerate wound healing during tissue repair. Previous studies suggest that these factors stimulate the proliferation of fibroblasts and thus the accumulation of scar tissue at the wound site, a proposed necessary component in the tissue repair process. Recent studies suggest that multipotent mesenchymal stem cells rather than fibroblasts compose the majority of the initial non-lymphocytic granulation tissue at the wound site. The current study was undertaken to determine the proliferative, progressive, and/or differentiative activities of PDGF-AA, PDGF-BB, and PDGF-AB on a clonal population of mouse pluripotent mesenchymal stem cells. Swiss-3T3-XYP mesenchymal stem cells were treated with a 0.0 to 500 ng/ml concentration range of these factors and assayed for cellular proliferation, progression, and commitment. The results show that PDGF-AA, PDGF-BB, and PDGF-AB stimulate an increase in cellular proliferation in these cells without affecting their subsequent progression, commitment or differentiation potential. These results suggest that PDGF may be used to stimulate proliferation of mesenchymal stem cells at the wound site and, in conjunction with non-fibrogenic differentiation factors, to stimulate normal tissue restoration rather than the obligatory scar tissue formation during tissue repair.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 326 EFFECTS OF BIO-ACTIVE FACTORS ON AVIAN LINEAGE- COMMITTED AND PLURIPOTENT MESENCHYMAL STEM CELLS, Henry E. Young, Shannon L. Pederson, Christina M. Klausmeyer, Jennifer C. Smith, Carmen C. Kavali, John J. Rogers, and Paul A. Lucas, Department of Surgery and Division of Basic Medical Science, Mercer University School of Medicine, Macon, GA 31207.

Previous studies have noted the presence of mesenchymal stem cells located within the connective tissue matrices of avian skeletal muscle, skin, atria, and ventricles. Clonal analysis coupled with dexamethasone treatment revealed the presence of multiple populations of mesenchymal stem cells composed of both lineage-committed stem cells and pluripotent stem cells. This study was undertaken to assess the effects of bio-active factors on the phenotypic expression of both populations of mesenchymal stem cells. Cells were isolated and cloned using conditions optimal for the isolation, cryopreservation, and propagation of avian mesenchymal stem cells. Cell aliquots of both lineage-committed stem cells and lineage-uncommitted pluripotent stem cells were plated at $2x10^4$ cells/well in 24-well 1% gelatinized tissue culture plates and examined using concentration ranges of recombinant-human growth factors, purified growth factors, and novel inductive agents, i.e., PDGFs, PD-ECGF, b-FGF, TGF-β, insulin, IGF-I, IGF-II, dexamethasone, BMP, MMP, and SIF. As noted, these bio-active factors effected the stem cells in either a stimulatory or inhibitory manner, with respect to three separate activities: proliferation, progression, or commitment. This study suggests the potential for using mesenchymal stem cells in conjunction with bio-active factors to effect tissue repair.

Supported by Rubye Ryle Smith Charitable Trust and the Clinical Research Center Fund of the Medical Center of Central Georgia.

H 327 TRANSPLANTATION OF CULTURED MURINE YOLK SAC CELLS INTO ALLOGENIC FETAL MICE, Jeung S. Yun, Michael A. Reed, Eric Holle, Linda Cioffi and Thomas E. Wagner. Edison Biotechnology Institute of Ohio University, Athens, Ohio 45701

The yolk sacs known to be progenitor cells for hematopoiesis eventually develop into blood cells in adult animals. Murine early yolk sacs from Balb/c and C57BL mice were cultured over 5-10 passages in a media containing leukemia inhibiting factor. Each strain of cultured cells were injected into fetuses of allogenic mice at the 7 to 14 day of pregnancy through the uteri of pregnant females. 152 animals out of 447 injected fetuses were born. 12% of adult Balb/c mice were positive for a surface marker MHC-1 H2b (clone:AF6-88.5) specific for C57BL mice in the lymphocytes of peripheral blood. Also, eight out of ten or 80% of deformed neonates tested positive for donor surface makers on spleen and liver tissues. Furthermore, the majority of lymphocytes in host animals seem to be donor cells with a range of 70% -95%. Other cell surface markers tested were , CD3 ε (clone; 145-2C11) and CD45 (Ly-5/B220) for T cell and B cell lymphocytes respectively.

We conclude that, because of the cell surface phenotype in lymphocytes, injected early embryonic yolk sac cells reconstituted the host animal and gave rise to T cells and B cells.

The present studies suggest that early cultured yolk sac cells can be injected into fetuses through the uteri of pregnant females to replace a potentially defective hematopoietic system of host animals. This application could be of possible use in human therapy.

Late Abstract

HEMATOPOIETIC DYSFUNCTIONS IN IL-6 DEFICIENT MICE.

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Interleukin-6 (IL-6) is a pleiotropic growth factor involved in inflammatory responses. Specific effects on nervous, hepatic and nephritic cells have been reported also. Different studies *in vitro* indicated an important role for IL-6 in the regulation of growth and differentiation of several hematopoietic lineages including lymphocytes, granulocytes, macrophages and megakaryocytes. Its role in controlling the cycling state of primitive hematopoietic cells by shortening the Go phase of quiescent precursors has been proposed. With the aim of dissecting the essential function(s) of IL-6 *in vivo*, both in adult and fetal hematopoiesis, we have studied knock-out mice deficient for this cytokine.

The phenotypic distribution of different lymphohematopoietic cell populations in thymus, spleen and lymph nodes, did not show any major abnormality in mutant mice. Only in the bone marrow (BM), significant differences in the levels of cells bearing surface markers for T and B lineages were detected. This effect was also observed in the BM of lethally irradiated wild type (wt) mice which were reconstituted with IL-6 deficient BM cells. In spite of the apparently normal phenotype of T-lymphocytes in these mice, spleen T-cells from IL-6 deficient mice showed a stronger proliferative response to ConA and superantigens (SEB, SEA) than wt littermates. The IL-6 deficiency resulted, however, in marked differences in the hematopoietic progenitor population. Thus, mutant mice showed a reduction of CFU-GM committed progenitors in the bone marrow, accompanied by an expansion of the same cell type in the spleen (3.5 fold). However, the absence of IL-6 did not seem to affect equally to other lineages, since erythroid precursors were expanded both in the bone marrow (23 fold) and in the spleen (5 fold). IL-6-deficient mice also showed a significant decrease in the number of more primitive multipotential progenitors (CFU-S 12d and pre-CFU-S) than wt mice. This was observed both when wt or IL-6 deficient mice were used as receptors for these assays. However, when mutant mice were used as receptors, the differences were more remarkable, suggesting diminished seeding capability in the splenic and bone marrow stromas of IL-6 deficient mice.

These data suggest an important role *in vivo* for IL-6 in the control of differentiation/proliferation of early hematopoietic precursors which could not be efficiently rescued by others interleukins in the absence of IL-6. Different experiments to address the implication of IL-6 in the maintenance *in vivo* of long-term reconstitution stem cells, self renewal capacity and cycling status are also underway.