HEMATOPOIESIS

Organizers: David Golde and Charles Sherr

February 27- March 5, 1992

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Keynote Address

M 001 HEXAMETHYLENE BISACETAMIDE AND RELATED AGENTS AS INDUCERS OF DIFFERENTIATION OF TRANSFORMED CELLS: MECHANISM OF ACTION AND POTENTIAL FOR CANCER THERAPY, Paul A. Marks[1], Victoria Richon[1], Richard A. Rifkind[1], and Ronald Breslow[2], [1]Memorial Sloan-Kettering Cancer Center, New York 10021 and [2]Columbia University College of Physicians and Surgeons, New York 10027.

Hexamethylene bisacetamide (HMBA) and related polar/apolar compounds induce various transformed cells to differentiate and cease proliferation. Our laboratory has employed inducer-mediated differentiation of murine erythroleukemia cells (MELC) as the primary model to study the mechanism by which these agents induce differentiation. Induction of terminal differentiation by these compounds appears to involve an initial effect on cell membrane structure with an alteration in the properties of membrane-associated proteins. PKC plays a role in HMBA induced signal transduction leading to terminal differentiation. There is a rapid translocation of protein kinase C activity from the cytosol to the membrane. There are several PKC isozymes in MELC, including α, ϵ, δ, but no β or τ. Inducer must be present during G_1/early S to cause commitment to terminal differentiation. The changes in PKC activity are followed by down-regulation of c-myc, c-myb and p53 proteins in essentially all cells. These changes are required for HMBA-mediated differentiation, but not sufficient, since removal of inducer stops recruitment to terminal differentiation. An increase in the level of under phosphorylated pRB protein is associated with prolongation of G_1 and eventual arrest of cells in G_1. Irreversible commitment to terminal cell division and active transcription of globin genes is first detected during the prolonged G_1 which follows the initial cell transit through G_1/early S in the presence of HMBA. Commitment increases stochastically with continued culture with inducer. We hypothesize that HMBA, through the PKC signaling mechanisms, causes early modulation in expression of a number of genes which are a prerequisite to, but not sufficient for, commitment. A subsequent stochastic event(s) occurring in G_1 determines commitment to terminal differentiation.

HMBA has been evaluated as a cytodifferentiation agent in phase I/phase II clinical trials. HMBA can induce partial and complete remission in patients with solid tumors, myelodysplastic syndrome and acute myeloid leukemia. However, HMBA is not an ideal differentiation inducer owing, in part, to dose-limiting toxicity. Related polar/apolar compounds have been developed which are >100 to >1000 fold more active than HMBA as differentiation inducers in vitro.

Hematopoietic Stem Cells

M 002 DIFFERENTIAL REGULATION OF PRIMITIVE NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS - A STARTING POINT FOR FUTURE BASIC AND CLINICAL INVESTIGATIONS. Eaves, C.J., Sutherland H.J., Udomsakdi C., Hogge D.E., Barnett M.J., and Eaves A.C. Terry Fox Laboratory, British Columbia Cancer Agency and University of British Columbia, Vancouver, B.C. Canada.

A population of primitive cells in normal human marrow with properties characteristic of murine long-term repopulating cells can be readily and reproducibly quantitated by assessment of their ability after a minimum of 5 weeks in culture with competent fibroblast feeders to have generated clongenic progenitors detectable in secondary methylcellulose assays. Cells with the same properties also circulate in the peripheral blood of normal individuals at a low frequency (~3 per ml). These "long-term culture-initiating cells" (LTC-IC) are, regardless of their origin, relatively well maintained in standard LTC-IC; i.e., during the first 7-10 days their numbers remain unchanged; thereafter, they slowly decline with a half-life of ~12 days. These two phases of different behaviour also suggest the operation of different regulatory mechanisms. For example, in the initial phase LTC-IC maintenance is indifferent to the presence of an adherent cell population that is, however, required during the second phase, and incubation of marrow cells under conditions that prevent or impede adherent layer formation has no deleterious effect on LTC-IC maintenance during the first 10 days. In contrast, after 10 days LTC-IC disappear much more rapidly in the absence of a suitable adherent feeder cell layer. The factors and/or interactions of feeder cells that rescue and/or stimulate LTC-IC in this second phase have not yet been identified, but production of IL-3, IL-6, G-CSF, GM-CSF or Steel factor does not appear to be required as shown by the ability of +/+ or Sl/Sl murine fibroblasts to effectively substitute for human marrow feeders in co-culture experiments using highly enriched LTC-IC starting populations. The LTC-IC assay can also be used to detect and quantitate an analogous Ph^1-positive cell type in patients with CML. These primitive leukemic cells have typically been found to be present in CML blood and marrow at elevated and decreased frequencies, respectively, by comparison to normal values. The average clonogenic cell-producing potential of these leukemic LTC-IC is normal, but their maintenance in LTC is highly defective. These latter studies suggest a novel consequence of bcr-abl activity and provide a theoretical basis for the design of clinical treatment protocols that may require or can exploit the use of cultured marrow autografts.

M 003 STUDIES IN CULTURE OF CELL-CYCLE DORMANT HEMOPOIETIC PROGENITORS IN MAN AND MOUSE, Makio Ogawa, Fumiya Hirayama, and Anne G. Leary, Ralph H. Johnson Department of Veterans Affairs and Department of Medicine, Medical University of South Carolina, Charleston, South Carolina.

Research in our laboratory continues to be focused on characterization of the early progenitors and the factors regulating their kinetics. In steady state marrow the early hemopoietic progenitors are dormant in cell cycle and divide infrequently to yield actively cell cycling multipotential progenitors. Earlier we reported that IL-3 supports proliferation of multipotential progenitors but that it does not trigger cell cycling of the dormant progenitors. We then identified a total of four synergistic factors, IL-6, G-CSF, IL-11 and c-*kit* ligand which enhance IL-3 dependent formation of murine blast cell colonies in part by shortening the dormancy period of the progenitors. Now we have obtained evidence that these synergistic factors can trigger the cell cycling of dormant human hemopoietic progenitors. We have isolated CD34+, HLA-DR- cells by two different approaches. Progenitors in this population were unable to form colonies in the presence of IL-3 alone but yielded a substantial number of colonies, including multilineage colonies, in the presence of a combination of IL-3 and either IL-6, G-CSF, IL-11, c-*kit* ligand or LIF/DIA. Studies with delayed addition of factors to the cultures indicated that this population of cells also requires IL-3 or GM-CSF to survive even while dormant. In contrast, none of the synergistic factors were able to replace IL-3 or GM-CSF in this function. *In vivo* studies of murine hemopoiesis provided strong evidence for lymphohemopoietic stem cells but it has not been possible to assay these cells *in vitro*. We have developed a two-step culture assay that supports clonal proliferation of lymphohemopoietic progenitors. Day 2 post 5-FU marrow cells were enriched for dormant progenitors. When single cells were plated in culture by micromanipulation in the presence of pokeweed mitogen-spleen conditioned medium (PWM-SCM), erythropoietin, c-*kit* ligand and IL-7, 43% of the single cells formed primary myeloid colonies. Individual colonies were then picked, washed and replated in methylcellulose culture containing c-*kit* ligand and IL-7. 43% of the primary colonies gave rise to lymphoid colonies in secondary culture. Cells of the lymphoid colonies were blast-like and B220+, sIg-, Mac-1-, Ly-1-, L3T4-, Lyt-2-, CD3-. 30% to 70% of the cells were Thy-1+. Mu chain mRNA was detected in most of the cells by *in situ* hybridization with antisense RNA probe. When lymphoid colonies derived from a single cell were pooled and injected into scid mice, donor-type IgM rose in the serum of mice for 3 months and spleens contained mature B cells. These results indicated that most cells in the lymphoid colonies were pre-B cells and established clonal proliferation of lymphohemopoietic progenitors in culture. These results also indicated that significant numbers of the cell-cycle dormant progenitors in the post 5-FU marrow cells which were considered to have only myeloid potentials also possess B-cell capabilities.

B Lymphocytes

M 004 TRANSLOCATIONS TO TRANSGENESIS: INSIGHTS FOR NORMAL AND MALIGNANT HEMATOPOIESIS, Suzanne Cory, Andreas Strasser, Andrew Elefanty, Ygal Haupt, Alan Harris and Jerry M Adams, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.

The chromosome translocations associated with certain cancers hallmark the genetic changes that precipitate the disease. The first to be defined at the molecular level was the translocation of c-*myc* to the immunoglobulin heavy chain locus which typifies most Burkitt lymphomas and murine plasmacytomas. Definitive proof that the *myc*/Ig translocation is cancer-inducing was provided by transgenic mice expressing c-*myc* linked to the 5′ IgH enhancer (termed Eμ) (1). All Eμ-*myc* mice develop disseminated clonal pre-B or B lymphomas which are preceded by a benign hyperproliferation of pre-B cells. Although it is clear that tumor onset requires somatic mutation in addition to transgene expression, identification of these changes has proven a difficult task. Recent efforts to identify these changes will be summarised.

The gene translocated to the IgH locus in another B lymphoid tumor, follicular lymphoma, is *bcl*-2, which encodes a membrane-associated protein apparently localized to mitochondria. The primary role of *bcl*-2 in neoplasia appears to be to promote cell survival, as first revealed by *in vitro* experiments in which it was shown that constitutive high expression of *bcl*-2 permitted factor dependent cells to survive the withdrawal of factor (2). Our recent studies with transgenic mice developed to express *bcl*-2 constitutively in either B or T lymphoid cells (3,4) have provided intriguing insights about the function of this novel gene and its potential role in normal lymphopoiesis.

The translocation product in chronic myeloid leukemia encodes a hybrid protein, *bcr-abl*, which has enhanced tyrosine kinase activity compared to c-*abl*, and this is believed to be the key factor initiating disease. Transgenic approaches to creating a murine model for CML have had limited success. In an alternative approach, we have reconstituted lethally irradiated mice with bone marrow cells infected *in vitro* with a *bcr-abl* retrovirus (5). A variety of myeloid and lymphoid diseases was observed, often within a single animal. The relative incidence of the various tumor types depends on the mouse strain, the infection protocol and, probably, the retroviral LTR. Intriguingly, *bcr-abl* cell lines were found to frequently switch from mast to erythroid/megakaryocytic differentiation *in vitro* and one exceptional line became granulocytic.

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5. Elefanty, A.G., Hariharan, I.K. and Cory, S. EMBO J. **9**, 1069-1078, 1990.

M 005 BCL-2: AN ANTIDOTE TO PROGRAMMED CELL DEATH, Stanley J. Korsmeyer, David Hockenbery, Charles Sentman, Gabriel Nunez, and Tim McDonnell, Howard Hughes Medical Institute and the Departments of Medicine, Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. The t(14;18) chromosomal breakpoint found in follicular B cell lymphoma juxtaposes the new proto-oncogene, Bcl-2, with the Ig heavy chain gene resulting in the marked over production of Bcl-2. Subcellular localization studies indicated that Bcl-2 was novel among proto-oncogenes being a 25 kD integral inner mitochondrial membrane protein. Moreover, it displays the unique role of blocking programmed cell death without promoting cell division. Early hematopoietic cells deprived of cytokines (IL-3, GM-CSF or IL-4) die by apoptosis, in which plasma membrane blebbing and volume loss is followed by nuclear condensation and an endonucleolytic cleavage of DNA into oligonucleosomal length fragments. Overexpression of Bcl-2 blocks this death program.

Prolonged Cell Survival is Oncogenic. Transgenic mice bearing a Bcl-2-Ig minigene that recapitulates the t(14;18) initially display a polyclonal expansion of resting B cells with a prolonged life-span. Over time these mice develop monoclonal, high grade lymphomas which often possess a c-myc translocation. Extended cell survival appears to be a key event which favors the acquisition of secondary genetic events.

Maintenance of B Cell Memory Represents a Normal Physiologic Role for Bcl-2. Bcl-2-Ig mice which overproduced Bcl-2 provided an *in vivo* model to assess the role of Bcl-2 upon immune responsiveness. Secondary immune responses by these transgenics were markedly protracted. This resulted from long-term persistence of antibody secreting plasma cells as well as extended lifetime for resting memory B cells. Bcl-2 spared the need for antigen in maintaining immune responsiveness. One normal role for Bcl-2 appears to be the generation and maintenance of B cell memory.

Bcl-2 Inhibits Multiple Forms of Apoptosis but not Negative Selection within Thymocytes. In the thymus, Bcl-2 is present in the mature T cells of the medulla but only rare cells within the cortex. To assess the role of Bcl-2 in the programmed death of thymocytes, lck^{Pr}-Bcl-2 transgenic mice were generated that redirected Bcl-2 expression to cortical thymocytes. Bcl-2 protected immature $CD4^+8^+$ thymocytes from glucocorticoid, radiation, as well as anti-CD3 induced apoptosis Moreover, Bcl-2 alters T cell maturation resulting in an increased percentage of $CD3^{hi}$ and $CD4^-8^+$ thymocytes. Despite this, clonal deletion of T cells which recognize endogenous superantigen still occurred. Multiple death pathways operate within the thymus that can be distinguished by their dependence on Bcl-2. Thus, Bcl-2 constitutes the first member of a new class of oncogenes: regulators of programmed cell death. It functions in an autosomal dominant fashion as an antidote to apoptosis. Extended cell survival is oncogenic.

M 006 AN ANTISENSE OLIGONUCLEOTIDE COMPLEMENTARY TO A SEQUENCE Iγ2B IS A POTENT B CELL MITOGEN, William E. Paul, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

In an effort to determine whether the germline Iγ2b transcript played some direct function in switching of B cells to the expression of immunoglobulin of the γ2b H chain class, B cells treated with lipopolysaccharide (LPS) were incubated with a phosphorothioate antisense oligonucleotide complementary to positions 160-177 in the Iγ2b exon. The oligonucleotide was a potent inhibitor of IgG2b production; however, it also inhibited secretion of Ig of all isotypes by 90 to 95%. Rather than diminishing the steady state level of the germline γ2b transcript, the oligonucleotide caused a 10 to 20 fold increase in the expression of this transcript as measured by both Northern and RNase protection assays. The increase in steady state mRNA levels was not associated with a prolongation in its half life. B cells treated with the oligonucleotide were stimulated to synthesize DNA and to proliferate. In addition, the antisense oliognucleotide was a potent enhancer of responses to other B cell mitogens but it had no effect on T cells nor on cell lines of T, myeloid or mast cell origin. Limiting dilution analysis suggested that the antisense acted directly on the B cells. Furthermore, the antisense oligonucleotide also caused increases in levels of class II MHC expression on all B cells, suggesting that most B cells responded to the oligonucleotide. The use of a series of other phosphorothioate oligonucleotides, including a set of "mutants," indicated that the effect was specific and that the capacity of oligonucleotides to mediate mitogenisis was correlated with their capacity to hybridize with ^{32}P-labelled sense oligonucleotide. These results strongly suggest that the target sequence of the antisense oligonucleotide is the Iγ2b sequence chosen. Whether the antisense oligonucleotide mediates its effect by interacting with sequences in the mRNA or in the gene has not been established nor has the relationship of the overexpression of the germline γ2b transcript to the B cell mitogenic effect.

T Lymphocytes

M 007 LYMPHOCYTES FUNCTIONS AND ONTOGENY IN GENE-TARGETTED MUTANT MICE,
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T lymphocytes recognize their antigen peptides and Major Histocompatibility Complex products with the use of their T cell antigen receptors (TcR). In addition to the α and β chains of TcR, the interaction between T cells and their target cells or antigen presenting cells is also assisted by a series of other cell surface polypeptides. Most notable of these are CD4 and CD8, which are selectively expressed on mature helper/inducer and killer/suppressor T cells, respectively. Upon engagement of their ligands, a series signals are being transduced intracytoplasmically via some of these molecules and their associated proteins. Perhaps the most important enzyme in this signal transduction process is the lymphocytes specific tyrosine kinase lck. In an attempt to gain better understanding on the roles of these molecules in T lymphocyte functions and ontogeny, we generated a series of mutant mice with disruptions in these genes. These mutant mice are being analysed in order that we can evaluate the importances of these genes in T cell development.

In addition to studying development, the roles of these molecules in autoimmune diseases, transplant rejection and tumor injection can also be analysed in the appropriate experimental mouse strains carrying mutations of these genes.

M 008 ROLE OF THE *lck* TYROSINE PROTEIN KINASE IN T CELL ACTIVATION,
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Stimulation of T lymphocytes through the antigen receptor induces increased tyrosine protein phosphorylation, elevated free cytoplasmic calcium, and activation of protein kinase C. T lymphocytes that do not express the cell surface tyrosine protein phosphatase CD45 show none of these changes after stimulation. This suggests that tyrosine dephosphorylation must precede increased phosphorylation of proteins on tyrosine, elevation of cytoplasmic calcium, and activation of protein kinase C during T cell activation. Of interest therefore is the identity of the substrate or substrates of CD45.

The *lck* tyrosine protein kinase is one apparent substrate of CD45 in the SAKR murine T cell line. It is phosphorylated to a 3 fold greater extent at a site of inhibitory phosphorylation in a variant of the SAKR T cell line that does not express CD45. CD45 therefore has the properties of a natural activator of the *lck* kinase.

We are interested in the generality and specificity of the interaction of CD45 and the *lck* kinase. T lymphocytes express a number of tyrosine protein kinases besides $p56^{lck}$. One of these is the *fyn* protein, a 59,000 dalton tyrosine protein kinase that is a close relative of $p56^{lck}$. Because it can be recovered in association with the T cell receptor if cells are lysed under gentle conditions, $p59^{fyn}$ may play a role in T cell activation. We have examined the phosphorylation of the *lck* and *fyn* protein kinases in three lines of CD45-negative T cells. The *lck* kinase was phosphorylated to an increased extent at the inhibitory site in all three cell lines. In contrast, the phosphorylation of the *fyn* kinase was unaltered in CD45-negative cells. This suggests that CD45 exhibits considerable polypeptide substrate specificity. Additionally, these results are consistent with the idea that it is the dephosphorylation of the *lck* kinase by CD45 that is critical in signal transduction during T cell activation.

M 009 T CELL ANTIGEN RECEPTOR SIGNAL TRANSDUCTION AND ITS REGULATION,
Arthur Weiss[1,2], Bryan Irving[2], Andrew Chan[2], and Gary Koretzky[3], Howard Hughes Medical Institute[1], University of California[2], San Francisco, Ca 94143, University of Iowa[3], Iowa City.

The T cell antigen receptor (TCR) is an eight chain oligomeric complex involved in antigen recognition and in signal transduction. Recent studies from our lab using chimeric receptors have demonstrated that the cytoplasmic domain of the TCR ζ chain serves to couple the TCR to intracellular signalling mechanisms. Thus, a chimeric CD8/ζ receptor can induce the early, protein tyrosine kinase and phospholipase C (PLC), and late events, interleukin-2 production, characteristic of TCR stimulation. In order to define the mechanism by which the ζ chain subserves this function we have begun to study the domains of ζ that are involved in signal transduction and to study proteins that interact with the ζ chain. The function of such chimeras correlates well with their ability to interact with and activate a protein tyrosine kinase and also associate with a 70 kilodalton tyrosine phosphoprotein (ZAP-70) following receptor stimulation. Although the identity of the tyrosine kinase that is regulated by the ζ chain is not known, it is likely to play a pivotal role in TCR signal transduction. Activation of this kinase and the resultant tyrosine phosphorylation of PLC γ1 is likely to be the means by which the TCR activates PLC catalytic activity in vivo. Interesting concepts regarding the regulation of this TCR associated kinase are also emerging from studies with CD45 tyrosine phosphatase deficient cells. In such cells, stimulation of the TCR fails to activate the TCR regulated kinase. A model linking this tyrosine kinase and the CD45 tyrosine phosphatase in TCR signal transduction will be discussed.

Myeloid Growth Factors

M 010 THE 'HELIX-LOOP-HELIX' GENE SCL AND ITS ROLE IN HEMOPOIETIC DIFFERENTIATION

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The SCL gene is a member of the 'helix-loop-helix' (HLH) family of transcription factors and was first identified because of its involvement in a 1;14 translocation in a stem-cell leukemia.

SCL is predominantly expressed in erythroid tissues and cell lines and is also expressed in normal mast cells, mast cell lines and megakaryocyte cell lines. However despite its involvement in up to 25% of T-cell acute lymphoid leukemias, SCL is not normally expressed in T-cells or T-cell lines. Thus SCL is expressed in the same lineages as GATA-1 a previously isolated hemopoietic transcription factor. SCL and GATA-1 are also coexpressed in IL-3 dependent myeloid cell lines and in murine erythroleukemia cells where they undergo coordinated biphasic modulation, with late increase, during chemically-induced erythroid differentiation. The kinetics of SCL and GATA-1 expression are inversely correlated with changes in ID (a "negative" HLH protein) and are distinct from changes in MYC, MYB and erythropoietin receptor transcripts. During induced myeloid differentiation of K562 cells, SCL and GATA-1 also undergo coordinate changes with an appropriate late decrease in expression. In non-hemopoietic tissues SCL is detected in adult and developing brain (where GATA-1 is not reportedly expressed): in post-mitotic neurons of metencephalon and roof of mesencephalon (in day 14.5 embryos).

An antisense SCL strategy was also employed to examine the role of SCL in K562 cells (in which SCL is normally expressed). Clones electroporated with antisense SCL vector grew more slowly than control cells. Non specific toxicity was excluded using Raji cells (that do not express SCL). Reduced growth was due to both a doubling of cell cycle time and 50 fold reduction in self-generation in antisense clones. Thus SCL promotes self-renewal in K562 cells and is therefore implicated in proliferation and differentiation events in normal erythroid cells.

M 011 MODULATION OF HEMATOPOIESIS WITH PIXY 321 - A GENETIC FUSION PROTEIN OF GM-CSF AND IL-3,

Steven Gillis[1], Thomas MacVittie[2], Saroj Vadhan-Raj[3], Steve Reed[4], Ronald Hoffman and Doug Williams[1], [1]Immunex Corporation, Seattle, WA 98101, [2]Armed Forces Radiobiology Institute, Bethesda, MD, [3]MD Anderson Cancer Center, Houston, TX, [4]Seattle Biolmedical Research Institute, Seattle, WA, [5]University of Indiana Cancer Center, Indianapolis, IN.

PIXY 321 is a molecular hybrid molecule consisting of GM-CSF and IL-3 cDNA(s) joined via a serine/glycine leader sequence. The protein when expressed in yeast (using cDNA(s) in which n-linked glycosylation sites and kex-2 protease cleavage sites have been altered contains O-linked sugars and migrates at a molecular weight of 31kd on SDS polyacrylamide gels. In vitro, PIXY 321 has been shown to exhibit enhanced specific activity as compared to equivalent concentrations of IL-3 and GM-CSF, regardless of whether the assay used to demonstrate such acitivity measured AML blast proliferation, CFU-GM, CFU-GEMM or BFU-E production. In vivo administration of PIXY 321 to normal primates resulted in a marked enhancement of both neutrophil and platelet production. Treatment of primates whose hematopoietic systems had been damaged either by radiation or chemotherapy with PIXY 321 also resulted in enhanced neutrophil and platelet recovery. In vivo administration of PIXY 321 was also found to significantly enhance neutrophil activation. Combination cytokine culture experimentation has shown that the combination of Steel factor with PIXY 321 is superior to any other cytokine combination tried in terms of stimulating marked in vitro proliferation of CD34+ cells, CFU-GM or CFU-GEMM regardless of whether the starting population for such cells is taken from peripheral blood or bone marrow. PIXY 321 stimulation of macrophages infected with a monocytotropic strain of HIV, resulted in marked diminution of viral pathogenesis, P24 production and giant cell formation. Phase I clinical trials of PIXY 321 have recently begun. Preliminary data from these trials will be discussed if available. Possible cellular mechanisms which could account for the enhanced bioactivity of PIXY 321 over and above that witnessed in cases of co-stimulation with monomeric IL-3 and GM-CSF will be discussed.

M 012 EFFECTS of p210 BCR/ABL ON GM-CSF AND IL-3 SIGNAL TRANSDUCTION PATHWAYS.

James D. Griffin, Keiko Okuda, Ursula Matulonis, Ravi Salgia, Brian Druker. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

The chimeric gene produced as a result of the Philadelphia chromosome translocation produces a fusion protein, p210 BCR/ABL, with elevated tyrosine kinase activity. There are several observations which suggest that some of the biological effects of BCR/ABL overlap with those normally regulated by IL-3. Introduction of BCR/ABL into murine IL-3-dependent cell lines induces factor-independent proliferation. Also, expression of either BCR/ABL or an IL-3 cDNA in murine marrow cells induces a similar myeloproliferative syndrome when the cells are transplanted in vivo. However, the mechanisms involved in this apparent overlap of function are unknown. In all factor-dependent cells examined, IL-3 rapidly activates a receptor-associated tyrosine kinase and induces transient, dose-dependent tyrosine phosphorylation of multiple substrate proteins. This is followed by phosphorylation and activation of other signal transducing molecules, including the serine/threonine kinases Raf-1 and MAP2 kinase. In order to investigate possible effects of p210BCR/ABL on IL-3 signal transduction, we generated sublines of murine IL-3-dependent cell lines 32Dcl3 (34 lines) or FDCP1 (3 lines) which express human p210BCR/ABL. The cell lines became factor-independent to a degree which depended on the amount of p210BCR/ABL expressed. The p210BCR/ABL (+) cell lines had constitutive tyrosine phosphorylation of several proteins which were transiently tyrosine phosphorylated in response to IL-3 in the parent cell line or control transfected cells. Prominent among these were p93, p70, and in some lines, p42. Also, expression of p210BCR/ABL inevitably led to hyperphosphorylation and activation of Raf-1. Expression of BCR/ABL also induced tyrosine phosphorylation of several novel proteins in all lines examined, including proteins of 120, 62,60, and 39 kDa. The p21ras GTPase activating protein (rasGAP) was identified as the major component of the tyrosine phosphorylated p120 species using specific anti-rasGAP antisera. Two rasGAP associated proteins, p62 and p190, were also constitutively tyrosine phosphorylated in p210BCR/ABL (+) cell lines. rasGAP, p62 and/or p190 were also tyrosine phosphorylated in the human p210BCR/ABL (+) cell lines K562, NALM-1, BV-173, and in primary blast cells from 8/10 patients with CML blast crisis. Tyrosine phosphorylation of rasGAP was not detected in normal myeloid cells or p210BCR/ABL (-) leukemic cell lines. rasGAP, p62, and p190 were found to co-immunoprecipitate with p210BCR/ABL in the 32D clones examined and in K562 cells. These results suggest that BCR/ABL has multiple effects on IL-3 signal transduction, including the induction of constitutive tyrosine phosphorylation of proteins normally transiently tyrosine phosphorylated in response to IL-3, causing hyperphosphorylation of Raf-1, and on inducing tyrosine phosphorylation and complex formation with rasGAP and associated proteins. The latter activity of p210 BCR/ABL kinase could indirectly activate another known IL-3 signal transduction pathway IL-3 (p21ras activation).

Myeloid Growth Factors II

M 013 THE MOLECULAR PHYSIOLOGY OF MYELOPOIESIS, Judith C. Gasson, Departments of Medicine (Hematology-Oncology) and Biological Chemistry and Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA.

The proliferation and maturation of myeloid progenitor cells are linked in a way that is not yet well understood. Our interest is to characterize the molecular events that are involved in both the proliferative response and the maturation of granulocytes and monocytes. GM-CSF is a cytokine that stimulates proliferation of myeloid progenitor cells to yield mature neutrophils, monocytes and eosinophils both *in vivo* and *in vitro*. GM-CSF is produced by a number of cell types in response to various immune stimuli. We have used recombinant promoter constructs to identify regions of the GM-CSF promoter responsible for inducible transcription of this gene in T-lymphocytes, as well constitutive transcription in fibroblast cell lines. A direct repeat of the sequence, CATT(A/T), is required for expression of this gene in all cell types examined. Biochemical characterization of nuclear factors interacting with this sequence is currently in progress. GM-CSF can also be produced in myeloid leukemia cells, which also express GM-CSF receptors and are responsive *in vitro*. Using recombinant GM-CSF promoter constructs, we have identified a potent cell type-specific negative regulatory region in the GM-CSF gene that is active exclusively in myeloid cell lines.

GM-CSF exerts multiple biological effects through binding to a high-affinity receptor (Kd 20-50 pM). Recently, molecular clones encoding both chains of the GM-CSF receptor have been isolated (Gearing et al., *EMBO J* 8:3667-3676, 1989; Hayashida et al., *PNAS* 87:9655-9659, 1990). Both the alpha and beta subunits of the GM-CSF receptor are members of the newly described cytokine receptor superfamily. In order to characterize the role of the conserved regions of the GM-CSF receptor, found in all members of the cytokine receptor superfamily, site-directed mutants have been prepared and analyzed for ligand binding and internalization. In order to more clearly understand the signal transduction processes involved in the initiation of a proliferative response to GM-CSF, we used the induction of immediate early response genes as an endpoint. The EGR-1/TIS8 gene is induced rapidly and transiently in response to GM-CSF, both in proliferating and terminally differentiated myeloid cells. To determine the precise nuclear factors interacting with the promoter, we prepared recombinant constructs of the human EGR-1/TIS8 promoter driving expression of a reporter gene. When these recombinant constructs are transiently transfected into the human factor-dependent cell line, TF-1, sequences regulating induction of EGR-1/TIS8 in response to GM-CSF, IL-3 and erythropoietin can be compared. These types of experiments will help to elucidate the signal transduction pathways utilized by factors that act to stimulate proliferation in myeloid cells through binding to receptors that are members of the cytokine receptor superfamily.

Terminal differentiation of myeloid cells requires expression of a complex array of structural genes necessary for the differentiated phenotype of granulocytes, monocytes and erythroid cells. Previously, several laboratories investigated the potential role of homeobox-containing genes in the lineage-specific differentiation of hematopoietic cells. We have been interested in the potential role of the gene, Hox 2.3, in regulating terminal differentiation of monocytes. To this end, we have examined the expression of the human Hox 2.3 gene in various human myeloid cells and cell lines. In addition, we have used anti-sense oligonucleotides during the induction of differentiation of bipotent myeloid cell lines. Results of these experiments further support the potential role of this gene in terminal differentiation of monocytic cells.

M 014 REGULATION OF MEGAKARYOCYTOPOIESIS, Jerome E. Groopman, Harvard Medical School, New England Deaconess Hospital, Boston, MA 02215

Studies of human megakaryocytopoiesis have been limited by technical difficulties in obtaining sufficient numbers of megakaryocyte progenitors and marrow megakaryocytes. Recently, several authentic permanent human megakaryocytic cell lines have been derived. These cell lines provide useful models to study cells of this lineage. Furthermore, techniques utilizing immunomagnetic beads have yield highly purified populations of marrow megakaryocytes. We have studied several issues related to regulation of megakaryocytopoiesis using both permanent cell lines as well as marrow megakaryocytes. Initial experiments suggest that human megakaryocytic cells are capable of producing a wide repertoire of cytokines including interleukin-1 and interleukin-6. These cytokines have previously been reported to potentiate megakaryocytopoiesis in vitro, so that an autocrine loop may be operative in this lineage. The c-kit protooncogene product, which serves as a receptor for the kit ligand/stem cell factor (KL/SCF), is expressed on the surface of megakaryocytic cell lines as well as bone marrow megakaryocytes. KL/SCF can augment numbers of megakaryocytic progenitors (CFU-MK) in semisolid culture in combination with other growth factors such as interleukin-3 and GM-CSF. KL/SCF also modestly increases proliferation of marrow megakaryocytes. It appears that the marrow megakaryocyte can utilize its c-kit receptor in adhesive interactions with the transmembrane form of KL/SCF present on marrow stromal fibroblasts. There is redundancy in the system in that other surface adhesive molecules on megakaryocytes contribute to the interaction with stromal fibroblasts. Of note is the observation that upon adhesion to bone marrow fibroblasts, a growth signal is transduced in the megakaryocytic cells. Experiments utilizing murine hematopoietic fibroblasts from steel mice that have a deletion in the KL/SCF locus have been performed. There is no adhesion or growth of human megakaryocytic cells cocultured with the steel fibroblasts. Steel fibroblasts that have been transfected with a construct expressing the transmembrane form of human KL/SCF can mediate adhesion with human megakaryocytic cells and transduce a growth signal. KL/SCF may serve several roles in regulation of megakaryocytopoiesis.

M 015 FUSION PROTEINS OF HEMATOPOIETIC GROWTH FACTORS: CHARACTERISTICS AND CLINICAL POTENTIAL, Linda S. Park, Unja Martin, Eric Jeffery, Joseph Dunn, and Douglas E. Williams, Immunex Corporation, Seattle, WA, 98101

Hematopoiesis is regulated by a complex network of cytokines which mediate the proliferation and/or differentiation of hematopoietic progenitor cells. While the clinical utility of two hematopoietic growth factors (GM-CSF and G-CSF) has been demonstrated, both *in vitro* and *in vivo* studies have shown that combinations of cytokines are more effective than single agents at stimulating progenitor cell growth. Optimal clinical utilization of hematopoietic growth factors is therefore likely to require use of a cocktail of two or more cytokines. One approach to combination therapy is the generation of hybrid cytokine molecules in which two cytokines are joined via flexible linker sequences which allow for proper folding of each component. This approach has been utilized to engineer a human GM-CSF and IL-3 fusion protein termed PIXY321. *In vitro* experiments have shown that both the GM-CSF and IL-3 components of the molecule are active, and *in vivo* administration to immunocompromised primates elicits both GM-CSF and IL-3 responses (enhanced neutrophil and platelet recovery, respectively). Unexpectedly, *in vitro* assays also showed that PIXY321 exhibited enhanced proliferative and hematopoietic colony-stimulating activity compared with either IL-3 and/or GM-CSF alone. A control fusion protein containing two linked molecules of human GM-CSF did not show a similar enhancement relative to GM-CSF alone. The receptors for human GM-CSF and IL-3 are known to contain specific low affinity binding subunits (α chains) and a shared β subunit. The interaction of PIXY321 with cells capable of binding both GM-CSF and IL-3 generates binding characteristics distinct from those of GM-CSF or IL-3. Data will be presented which suggests that the GM-CSF and IL-3 receptors may contain additional subunits (perhaps shared), and the enhanced specific activity of PIXY321 may be a function of its ability to interact with or generate a unique GM-CSF/IL-3 receptor complex. It has recently been shown that either IL-3 or GM-CSF can synergize with Steel Factor (MGF, SCF) to produce a modest expansion of progenitors from a human peripheral stem cell harvest. A greatly enhanced synergy is observed when MGF is combined with PIXY321. Hybrid proteins of Steel Factor combined with either GM-CSF or IL-3 have now been constructed with a flexible linker similar to PIXY321. *In vitro* experiments have shown that both components of these fusion proteins are active and investigations are underway to determine whether the hybrid molecules will exhibit enhanced biological activities relative to a mixture of their component cytokines. In contrast to the case of PIXY321, the receptor for MGF (c-*kit*) is not apparently related to the receptors for GM-CSF or IL-3. Studies with these new hybrid proteins should help clarify the role of shared receptor subunits and complex formation in the enhanced activity of PIXY321, both in the presence and absence of MGF. Other hybrid cytokine combinations will also be discussed.

Myeloid Growth Factors III

M 016 BIOLOGY AND ACTION OF COLONY STIMULATING FACTOR-1,
E. Richard Stanley[1], Wieslaw Wiktor-Jedrzejczak[2], Jeffrey W. Pollard[1], Joan S. Hunt[3], Orin Chisholm[1] and Laura K.H. Price[1], [1]Albert Einstein College of Medicine, Bronx, New York 10461, [2]Post Graduate Education Medical Center, CSK WAM, Warsaw, Poland, and [3]University of Kansas Medical Center, Kansas City, Kansas 66103

Colony stimulating Factor-1 (CSF-1) humorally regulates mononuclear phagocyte production. Previous correlative studies suggested that locally produced uterine CSF-1 plays an important role in placental development. The discovery that the osteopetrotic (*op/op*) mutant mouse possesses an inactivating mutation in the CSF-1 gene provides us with a system in which to study local and humoral regulation by this growth factor. *op/op* mice are toothless, have increased bone mass, decreased body weight, reduced femoral cellularity with dramatically reduced numbers of osteoclasts and macrophages and one tenth the number of peripheral blood monocytes and peripheral macrophages in locations such as the pleural and peritoneal cavities. Restoration of circulating CSF-1 to normal or higher than normal concentrations, by daily subcutaneous injections of human recombinant CSF-1 from day 7, corrected the congenital osteopetrosis, toothless phenotype, osteoclast deficit and deficits in splenic and femoral macrophages, but only partially restored body weight. In contrast, pleural and peritoneal cavity macrophages were not corrected except by direct intra-cavity inoculation, indicating that circulating CSF-1 can stimulate bone marrow osteoclast and macrophage production but that local synthesis and action of the growth factor are important for certain mononuclear phagocyte populations.

The local production of CSF-1 by the uterine epithelium is associated with the local proliferation of uterine decidual cells and macrophages and of placental trophoblastic cells, all of which express the CSF-1 receptor. This endocrine regulated uterine CSF-1 synthesis may therefore play a critcal role in pregnancy. To investigate this possibility, mating experiments were performed with *op/op* mice. Matings between *op/op* females and fertile/*op/op* male mice fail to yield progeny and whereas *op/op* males were 85% as fertile as *op/+* males when mated to *op/op* females, *op/op females* were only 40% as fertile as *op/+* females when mated to *op/+* males and their fertility was compromised at the level of both pre- and post-implantation development. Consistent with a local role for maternal CSF-1 during pregnancy, the fertility of *op/op* mothers is not improved by restoration of circulating CSF-1 from day 7 of age. The basis of the partial correction of the maternal fertility defect in matings where fertile *op/op* males were replaced by *op/+* males is currently under investigation.

These and other studies indicate that the presence of only some CSF-1 receptor bearing cell populations is solely regulated by CSF-1. Furthermore, they indicate that CSF-1 regulates some of its target populations humorally and some locally. CSF-1 is a homodimeric glycoprotein that can be rapidly secreted from the cell or expressed in a biologically active form as a membrane-spanning protein at the cell surface. Recent studies from this laboratory have shown that the predominant form of secreted CSF-1 is a proteoglycan. Local regulation by CSF-1 may therefore differ depending on which form is expressed by the producing cell.

Cytokines/Microenvironment

M 017 POTENTIAL ROLE OF CYTOKINE RECEPTOR MODULATION IN HEMATOPOIETIC RECOVERY,
Joost J. Oppenheim[1], Ruth Neta[2], Claire Dubois[1], Jonathan Keller[3], and Francis Ruscetti[1], [1]LMI, BRMP, NCI-FCRDC, Frederick, MD 21702, [2]AFRRI, Bethesda, MD 20814, [3]PRI/DynCorp, NCI-FCRDC, Frederick, MD 21701.

Interleukin-1 (IL-1) and TNF increase the survival of mice following a lethal dose of radiation by promoting bone marrow (BM) recovery. We investigated the contribution of cytokine receptor modulation to the enhanced hematopoiesis induced by IL-1. There are two structurally distinct IL-1 receptors (IL-1R); Type I present on T cells, fibroblasts and endothelial cells and Type II present on B cells, granulocytes and hematopoietic progenitor cells. BM cells from normal mice express low numbers of IL-1R. In vivo administration of IL-1 markedly increases IL-1R expression on both mature and immature bone marrow cells. This receptor upregulation has the same kinetics as the radioprotective effect of IL-1. Pretreatment of mice with anti-type I IL-1R antibody (35F5), which has been shown to prevent the radioprotective effect of IL-1, also blocked IL-1 induced IL-1R expression on BM cells. However, this antibody did not directly bind and block IL-1 binding to the Type II IL-1R expressed on hematopoietic progenitor cells, suggesting that IL-1R up-regulation by IL-1 is indirect. Anti-IL-1R also blocks induction of G-CSF. G-CSF is about half as potent as IL-1 in inducing IL-1R on BM cells. It is therefore likely that G-CSF mediates some of the induction of IL-1R by IL-1. Administration of IL-1 also stimulates the hypothalamic-pituitary-adrenal axis and culminates in the production of glucocorticoids (GC). GC increase the expression of IL-1R on B lymphocytes, macrophages and fibroblasts. IL-1 mediated [illegible]tion of IL-1R on BM cells in vivo is decreased in adrenalectomized mice presumably due to the lack of GC. The combination of G-CSF and dexamethasone equal the capacity of IL-1 to increase IL-1R on bone marrow cells. In conclusion, IL-1 promotes hematopoietic recovery from radiation by inducing the production of other hematopoietic growth factors and by upregulating cytokine receptor expression.

Red Cells

M 018 REGULATION OF THE ERYTHROPOIETIN GENE,
Kerry L. Blanchard, Deborah L. Galson, Joachim Fandrey, Mark A. Goldberg and H. Franklin Bunn, Harvard Medical School, Boston.

The survival and terminal maturation of erythroid cells depends on the hormone erythropoietin (Epo) which is produced in the kidney and liver in a highly regulated manner. When mammals are subjected to hypoxia, plasma Epo levels can increase 1000 fold. We have previously demonstrated that the human hepatoma cell line, Hep3B, synthesizes large quantities of erythropoietin (Epo) in a regulated manner in response to hypoxia and cobalt chloride ($CoCl_2$)[1,2] and that this regulation occurs at the Epo mRNA level and is, to a large extent, transcriptional. We have developed a highly sensitive and accurate means of measuring Epo mRNA levels by competitive PCR.[3] When Hep3B cells are incubated in the presence of decreasing O_2 tension from 150 to 7 Torr, there is a progressive increase in Epo mRNA to a maximum of 150-fold at 5-6 hours. We have investigated the transcriptional regulation of the human Epo gene in Hep3B cells by transient transfection. Portions of the Epo promoter, ranging from 5.5 kb to 70 bp upstream of the cap site, were placed 5' to the promoterless gene of a reporter protein, firefly luciferase. Basal enzyme activity (21% O_2) was compared to that following exposure to hypoxia or cobalt. These experiments permitted the identification of the minimal promoter element necessary for basal and inducible expression of Epo. A fragment 150 bp 5' to the cap site conferred a 6-10 fold induction in response to 1% O_2 and cobalt. The addition of a conserved 150 bp Epo element 3' to the polyadenylation site[4] increased the induction to 100-150 fold, similar to the enhancement of expression of Epo mRNA. No increase in luciferase production was observed in a hepatoma cell line that fails to produce Epo. Thus the combination of 150 bp promoter and 3' elements appears to be necessary and sufficient for tissue specific induction of Epo expression by hypoxia and cobalt in Hep 3B cells.

References

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M 019 CONTROL OF GLOBIN GENE EXPRESSION, Y.W. Kan[1,2], Paolo Moi[2], Peter T. Curtin[1], Tohru Ikuta[2], and Judy Chang[1], [1]University of California, San Francisco, CA 94143, [2]Howard Hughes Medical Institute, San Francisco, CA 94143

The high level and tissue-specific globin gene expression is regulated by sequences within and flanking the globin genes. In the β-globin gene cluster, several DNA hypersensitive sites (HS) constitute the Locus Control Region (LCR). The developmental switch from embryonic to fetal and adult globin gene expression is believed to be associated with a series of protein-DNA interactions between the hypersensitive sites and the promoters and enhancers of globin genes. We have investigated the sequences within these hypersensitive sites that are important for protein interactions by *in vivo* and *in vitro* experiments and attempted to enhance globin gene expression in gene transfer experiments.

Transfection of K562 cells and transgenic mouse experiments have localized the activities of one of the hypersensitive sites (HS2) to a tandem repeat of an AP1 binding site. The left repeat is also a consensus sequence for NFE-2 binding. Deletion of this repeat completely abolishes its activity, whereas point mutations diminish its activity. *In vivo* footprinting revealed a number of protein DNA interactions. We studied several cell lines that expressed different proportions of ε-, γ-, and β-globin genes. K562, which expressed ε and γ; HEL, which expressed γ with a small amount of ε, and two different MEL human hybrids containing only human chromosome 11: A181γ, which expresses 90% gamma and 10% beta and A181β, which expresses 95% beta and 5% gamma. In all four cell lines, HS2 showed footprints in several consensus sequences, including the functionally important NF-E2/AP-1, GGTGG and GATA-1. However, within the HS2, the HEL, A181β and A181γ exhibited differences in protected patterns from K562. In HS3, which also contains an NF-E2 and several GATA-1 and GGTGG motifs, K562 demonstrated no detectable protection and HEL only slight protection. In contrast, HS3 was prominently footprinted in both A181γ and A181β. In the β- promoter region, A181β showed protection, while A181γ did not. In the γ-promoter region, the CACC box of the cells that produced γ (e.g., K562, A181γ) was clearly protected while in A181β, the CACC box was not. Thus, the expression of different globin genes is associated with changing patterns of DNA-protein interactions. Interactions at HS2 are essential for the β- and β-like globin gene expression. Interactions at HS3 are found in cell lines which express little or no ε-globin, while interactions at the CACC box at the γ promoter are associated with γ-globin gene expression.

We utilized these sequences for enhancing globin gene expression in retroviral vectors. When the 400 bp of HS2 were incorporated in the retrovirus vectors, the globin gene sequences in all the packaging cell lines were rearranged. However, when only the core NFE-2/AP1 sequence was used, a large proportion of these packaging cell lines produced unrearranged virus. The production of β-globin mRNA was doubled with the addition of the NFE-2/AP1 sequence. Thus, the identification of the elements required for enhancement of β-globin gene expression may be useful for designing gene transfer vectors.

Signal Transduction

M 020 THE ROLE OF THE ERYTHROPOIETIN RECEPTOR IN MULTI-STAGE FRIEND VIRUS-INDUCED ERYTHROLEUKEMIA, Mark O. Showers, Jean-François Moreau, Alan D. D'Andrea, Dana-Farber Cancer Institute, Boston, MA.

The erythropoietin receptor (EPO-R), a member of the cytokine receptor superfamily, can be activated by binding either erythropoietin (EPO) or gp55, the Friend spleen focus-forming virus (SFFV) glycoprotein. The highly specific interaction of gp55 and EPO-R triggers cell proliferation and thereby causes the first stage of Friend virus-induced erythroleukemia. We have generated functional chimeric receptors of EPO-R and IL-3R, a related cytokine receptor which does not interact with gp55. Chimeric receptors that confer EPO-dependent proliferation are capable of binding gp55, but only those containing the EPO-R transmembrane region are activated by gp55. These results support a model in which the EPO-R contains two distinct regions which mediate gp55 interaction: a transmembrane activation domain and an extracytoplasmic binding domain. Also, activation of the EPO-R by EPO binding results in rapid, dose-dependent tyrosine phosphorylation of a 97 kD substrate in EPO responsive cells. Expression of the Friend Virus gp55 results in constitutive tyrosine phosphorylation of this same substrate, supporting the role of the substrate in the EPO-R signalling mechanism.

M 021 REGULATION OF SIGNAL TRANSDUCTION BY MYELOID COLONY STIMULATING FACTORS. Maribeth A. Raines[1], Marjan Daeipour[2], Andre Nel[2], John DiPersio[3], and David W. Golde[1], [1]Dept. of Medicine, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, [2]Dept. of Medicine, Division of Clinical Immmunology and Allergy, U.C.L.A., Los Angeles, [3]Dept. of Medicine, Hematology Unit, Univ. of Rochester, Rochester.

Myeloid colony stimulating factors such as GM-CSF and G-CSF promote the proliferation and differentiation of myeloid progenitor cells and enhance the function of mature effector cells. These pleiotropic effects are initiated by binding to their cell surface receptor. Although several members of the hematopoietin receptor superfamily have been cloned, their structure has not provided insight into the mechanism of signal transduction. We have examined the role of microtubule associated protein 2 (MAP2) kinase in GM-CSF and G-CSF-mediated signal transduction. MAP2 kinase is a serine/threonine kinase which has been extensively characterized in other cell systems. Although its activity is usually associated with mitogenic stimulation, our studies suggest that MAP2 kinase may play a role in regulating biological responses other than mitogenesis. Treatment of peripheral blood neutrophils or terminally differentiated HL-60 cells with GM-CSF induces a rapid and dose-dependent increase in MAP2 kinase activity. Maximal activity occurs within 5 minutes and the kinetics of the response varies depending on the target cell (prolonged in neutrophils and transient in neutrophilic HL-60 cells). GM-CSF appears to be one of the most potent inducers of MAP2 kinase activity identified to date producing a 7-fold and 4-fold increase in neutrophils and HL-60 cells, respectively. G-CSF, on the otherhand, does not affect MAP2 kinase activity in HL-60 cells and induces only 0.6-fold increase in activity in neutrophils. Tyrosine phosphorylation of MAP2 kinase, a 42 kDa protein (pp42), has been implicated as a potential regulatory mechanism and also appears to be important in GM-CSF-mediated signaling. GM-CSF induces tyrosine phosphorylation of a pp42 in both neutrophils and neutrophilic HL-60 cells. The kinetics of pp42 induction correlates with the induction of MAP2 kinase activity by GM-CSF. Immunoblotting with an antibody against rat MAP2 kinase suggests that GM-CSF induced pp42 is an activated form of MAP2 kinase. Tyrosine phosphorylation appears to be necessary for MAP2 kinase activation since its activity can be inhibited by treatment with the tyrosine kinase inhibitor, erbstatin analog. Taken together, our data support the notion that tyrosine phophorylation is critical to GM-CSF-mediated signal transduction and suggests that MAP2 kinase activation is an important biochemical event involved in GM-CSF signaling. MAP2 kinase may also prove to be central to the signal transduction pathways of other members of the hematopoietin receptor superfamily.

In addition to the membrane-bound form of receptors, many members of the hematopoietin receptor superfamily also express a soluble form. We have previously cloned and characterized a soluble form of the human GM-CSF receptor. The soluble GM-CSF receptor (sGMR) is a truncated form of the membrane-bound receptor (GMR) where 84 c-terminal amino acids (including the transmembrane domain) are replaced by 16 new amino acids. sGMR is a naturally occuring protein which results from alternate splicing of the GM-CSF receptor gene. The resulting protein is efficiently secreted and binds human GM-CSF with low-affinity. The function of soluble receptors and their role in regulating signal transduction by colony stimulating factors will also be discussed.

M 022 CSF-1 RECEPTOR SIGNALS REGULATE EARLY AND LATE TRANSITIONS DURING THE G1 PHASE OF THE CELL CYCLE, Charles J. Sherr[1,2], Hitoshi Matsushime[1,2], and Martine F. Roussel[1], Department of Tumor Cell Biology[1] and the Howard Hughes Medical Institute[2], St. Jude Children's Research Hospital, Memphis, TN 38105

CSF-1 is required throughout the G_1 phase of the macrophage cell cycle to stimulate proliferation and ensure cell survival, but once macrophages enter S-phase, they can complete cell division in the absence of the growth factor. CSF-1 deprivation leads to cell cycle arrest in early G_1, so that if transiently starved macrophages are restimulated, they enter the cell cycle synchronously. Such cells do not enter S phase unless CSF-1 is present throughout the entire G_1 interval. Therefore, CSF-1 minimally regulates steps at two cell cycle restriction points, temporally corresponding to both early and late G_1.

Stimulation of macrophages with CSF-1 activates the intrinsic protein tyrosine kinase (PTK) activity of the CSF-1 receptor (CSF-1R), initiating events that lead to induction of immediate early response genes. Studies of CSF-1R mutants that are defective in some, but not all, aspects of signal transduction indicate that receptor signals are simultaneously relayed through multiple pathways that act in concert to determine the magnitude and specificity of the biological response. When expressed in NIH/3T3 cells, a CSF-1R mutant containing a Phe for Tyr substitution at codon 809 exhibits nearly wild-type levels of ligand-dependent PTK activity and rapidly induces the expression *c-fos* and *junB*. However, cells bearing CSF-1R [Phe-809] are defective in their *c-myc* response and are concomitantly impaired in their ability to be cotransformed by ligand, to form CSF-1 dependent colonies in semisolid medium, and to grow in serum-free medium containing CSF-1. The enforced expression of *c-myc* in these cells restores their capacity for CSF-1 induced proliferation, thereby demonstrating a receptor-mediated bifurcation of signal transduction pathways during the immediate early response and underscoring the role of *c-myc* in G_1 progression.

Targets of the delayed early response to CSF-1 include a family of novel cyclins (*CYL*/Cyclin D). In macrophages, *CYL1* is induced in early G_1 and its mRNA and protein levels remain elevated throughout the cell cycle as long as CSF-1 is present. In contrast, *CYL2* is induced later in G_1, but its expression is periodic, being maximal at the G_1/S transition. Induction of *CYL* mRNAs by CSF-1 requires new protein synthesis, and the mRNAs and proteins are rapidly degraded when CSF-1 is withdrawn. *CYL*-coded proteins (p36) form complexes during G_1 with polypeptides that are immunologically related, but probably nonidentical, to the cell division cycle protein kinase, $p34^{cdc2}$. However, immune complexes containing $p36^{cyl1}$ lack histone H1 kinase activity. Possibly, certain of the $p36^{cyl}$ proteins might target cyclin-dependent kinases to other substrates or, alternatively, might negatively regulate kinase activity. Attractive substrates include the retinoblastoma gene product ($p105^{Rb}$), which normally functions to suppress S-phase entry and is presumed to be inactivated in late G_1 as a result of its phosphorylation by a *cdc2*-like enzyme. Another member of the *CYL* gene family (*CYL3*) is not expressed in macrophages, but has been detected in other hematopoietic cell lines. In human peripheral blood T lymphocytes, *CYL1* is not expressed; *CYL2* induction appears to be at least partially dependent upon IL-2 receptor activation; and *CYL3* is induced later in G_1 just prior to S-phase entry (J.D. Griffin and F. Ajchenbaum, personal communication). The nucleotide sequences of the murine *CYL* genes are more related to their human cognates than to each other, suggesting that *CYL* genes are unlikely to be redundant, but instead, may regulate distinct cell cycle transitions. The *CYL1* gene is a target of chromosomal rearrangement and amplification in certain tumors, implying that dysregulation of G_1 cyclins can contribute to oncogenesis.

Gene Transfer and Expression

M 023 THE ROLE OF TRANSCRIPTION FACTOR GATA-1 IN HEMATOPOIETIC CELL DIFFERENTIATION, Larysa Pevny[1], M. Celeste Simon[2], Elizabeth Robertson[1], Stuart H. Orkin[2] and Frank Costantini[1], [1]Dept. of Genetics and Development, College of Physicians & Surgeons, Columbia University, New York, NY, [2]Division of Hematology-Oncology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, and the Howard Hughes Medical Institute, Boston, MA.

GATA-1 is a zinc-finger transcription factor found in erythroid cells, which binds to GATA consensus elements in regulatory regions of the α and ß globin gene clusters, as well as many other erythroid cell-specific genes. GATA-1 is also expressed in megakaryocytes and mast cells, and thus it may have additional functions outside the erythroid lineage. To evaluate the role of GATA-1 expression in the development and differentiation of various hematopoietic cell types, we produced a murine embryonic stem (ES) cell line in which the sole copy of the X-linked GATA-1 gene was disrupted by gene targeting. We have examined the ability of GATA-1 deficient cells to participate in hematopoiesis by producing chimeric mice, as well as by studying the differentiation of the mutant ES cells in vitro. The results of these studies will be discussed.

M 024 CHICK ERYTHROID CELLS TRANSFORMED BY THE VIRAL MYB-ETS FUSION PROTEIN CAN BE INDUCED TO DIFFERENTIATE ALONG THE MYELOMONOCYTIC AND EOSINOPHIL PATHWAYS BY KINASE ONCOGENES, RAS AND TPA. Thomas Graf and Kelly McNagny, EMBL, Differentiation Programme, Meyerhofstr. 1, D-6900 Heidelberg, Germany

The avian retrovirus E26, which encodes a transcriptional activator-type oncoprotein containing both Myb and Ets domains, causes an acute leukemia in chickens which consists predominantly of early erythroid cells. We have shown earlier that neither oncogene alone can cause leukemia and that the two proteins have to be fused to be active in vivo(1). When the virus is used to infect 2 day old chick embryo cultures three types of transformed colonies are observed: early erythroid; myeloblast and mixed. Most of the mixed colonies are of clonal origin, suggesting that the E26 virus is capable of infecting uncommitted progenitors. Surprisingly, we have now found that the transformed erythroid cells themselves are multipotent. Thus they can be induced to differentiate into both myeloblasts and eosinophils if superinfected with oncogenic retroviruses encoding tyrosine kinases, serine kinases or Ras, or if treated with TPA. This process involves a) the complete extinction of erythroid markers, as well as of progenitor-specific cell surface antigens and b) the activation of a battery of myeloid/eosinophil markers, including the acquisition of specific growth factor dependence. The time period required varies with different clones, some clones showing a complete phenotypic change within 7 days, others requiring more than 10 days. In addition, the frequency of lineage commitment towards either the myeloid or eosinophilic lineage varies both with individual clones and the concentration of TPA used. Erythroleukemia cells transformed by E26 virus were also found to be multipotent. Our observations provide the basis of a new system to study the molecular basis of lineage commitment during hematopoiesis.

(1)Metz, T. & Graf, T. (1991) Fusion of the nuclear oncoproteins v-Myb and v-Ets is required for the leukemogenicity of E26 virus. Cell 66, 95-105.

M 025 MECHANISMS IN THE TRANSFORMATION OF HEMATOPOIETIC STEM CELLS, James N. Ihle, Kazuhiro Morishita, Takayasu Matsugi, Tetsunori Funabiki, Rudd Delwel and Brent Kreider, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

Interleukin-3 (IL-3) supports the proliferation and differentiation of early hematopoietic stem cells and cells committed to differentiation along the myeloid lineage. Cells committed to the granulocytic lineage acquire the ability to terminally differentiate in the presence of G-CSF while cells that commit to the erythroid lineage express receptors for erythropoietin (Epo) and require Epo for terminal differentiation. Transformation of IL-3 dependent progenitors in human and murine acute myelogenous leukemia (AML) eliminates the ability to terminally differentiate although Epo or G-CSF receptors are expressed and support proliferation in some cases. Moreover, the cells express GATA-1, a transcriptional factor that is required for erythroid lineage differentiation. This transformed phenotype is associated with activation, by retroviral insertion or translocations, of a zinc finger transcriptional factor gene, *Evi*-1. The *Evi*-1 gene product is a 145 kDa nuclear protein that contains an amino terminal domain of 7 fingers and a carboxyl domain of 3 fingers. In murine myeloid leukemias retroviral insertions immediately 5' to the gene or 90kb upstream of the gene activate expression. In human AML, the translocations that are associated with the activating the expression of the *Evi*-1 gene occur over a range of at least 300 kb surrounding the *Evi*-1 locus on 3q26. Consistent with a role for the *Evi*-1 gene in AML, introduction of the *Evi*-1 gene into myeloid cells that are capable of terminal differentiation, blocks this ability. To address the mechanisms involved, the sequence specificity of DNA binding has been determined for both the amino and carboxyl finger domains. Using the amino terminal domain of 7 fingers, a consensus binding sequence of GACAAGATAAGATAA was identified by binding of random 35mers. Consistent with this sequence, the amino terminal domain specifically bound to genomic fragments containing AGATA sequences, suggesting that the *Evi*-1 gene product may block differentiation by interfering with transcriptional factors such as GATA-1.

Clinical II

M 026 CLINICAL APPLICATIONS OF HUMAN GRANULOCYTE COLONY STIMULATING FACTOR. Janice Lynn Gabrilove.

The production of neutrophil granulocytes is a complex and dynamic process during which a small number of self-renewing stem cells give rise to lineage-specific progenitors that proliferate and mature in the bone marrow, subsequently entering the blood as mature polymorphonuclear leukocytes. One hematopoietic glycoprotein that appears to specifically control the survival, cycle activation, proliferation, and terminal maturation of the myeloid lineage is granulocyte colony-stimulating factor (G-CSF).

The major potential clinical applications for granulocyte colony factors lie in three general areas: 1) restoration of hematopoiesis by either preventing or accelerating recovery from iatrogenic-induced or disease related myelosuppression, 2) stimulation and production of functionally primed effector cells with anti-tumor capability and which are also able to augment general host defense, 3) clonal extinction of malignant disease differentiation induction alone or recruitment of cells into S-phase allowing them to be rendered more susceptible to killing by cycle specific agents.

The therapeutic evaluation of G-CSF growth factors is a rapidly moving field which holds great promise for the treatment of a variety of medical illnesses. In addition, an understanding of the mechanism and action of G-CSF should provide insight into its potential role in either clinical manifestations or pathophysiology of certain diseases. In this session, we will highlight specific clinical studies which have contributed so far to our understanding of the physiologic effects of G-CSF in man and its utility in the treatment of human disease.

Hematopoietic Stem Cells; B Lymphocytes

M 100 RAPID ENRICHMENT OF CD34+ PROGENITORS BY IMMUNOADSORPTIVE SELECTION.

C.N. Abboud, J.M. Winslow, J.L. Liesveld, and D.H. Ryan. University of Rochester Medical Center, Rochester, NY, 14642.

Anti-CD34 monoclonal antibodies recognize about 1% of human marrow cells, including primitive erythroid, myeloid and lymphoid progenitors. We have compared two selection methods (immunomagnetic beads and CEPARATE LC) to purification by FACS sorting of light density marrow. One immunoadsorption technique utilized immunomagnetic beads to isolate so-called lineage negative light density marrow (LDM) cells (Lin-). Goat anti-mouse IgG and goat anti mouse IgM beads (Advanced Magnetics) were incubated with bone marrow mononuclear cells stained with a cocktail of CD2, CD3, CD11b, CD15, CD16, CD19, CD71, and CD56. The lin- population was then stained with anti-CD34 and subjected to FACS sorting and analysis. The second immunoadsorption technique utilized CeprateTM LC, a column cell separation system (CellPro, Inc.). Light density marrow cells were stained with 12.8 (anti-CD34) moab followed by a biotinylated second antibody after which they were passed through avidin columns. Collected adsorbed cells were then analyzed by FACS utilizing fluorescent conjugated goat anti-mouse IgM. This avidin column technique resulted in populations which were 73+/-14% (n=9) CD34+ as per FACs analysis (range 52%-89%). CFU-GM and BFU-E per 100 cells were 13.1 ± 1.8 and 14.8 ± 2.8 in the adsorbed CEPRATE fraction, while unsorted LDM cells contained 0.5 ± 0.1 CFU-GM and 0.8 ± 0.2 BFU-E per 100 cells. In 4 nonparallel experiments utilizing magnetic bead selection of a Lin- population, corresponding numbers per 100 plated cells were 1.4 ± 0.4 CFU-GMs and 1.4 ± 0.4 BFU-Es. These results indicate that the progenitor populations generated by avidin-biotin column adsorption are at least ten-fold enriched for CFUs/BFUs and suggest that this method may be preferable to the more time-consuming magnetic bead selection method. We are presently using these cells to study CD38+ and CD38- cells in long-term marrow cultures.

M 101 TARGETED DISRUPTON OF THE *fyn* GENE.

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Although many of the biochemical events subsequent to T cell activation have been characterized, the mechanism of signal transduction from the receptor remains obscure. Previous studies suggest that *src* family protein tyrosine kinases may assist in this signalling process. In particular one form of p59*fyn* (the product of alternative splicing of primary *fyn* trasnscripts) has been shown to interact with the TCR complex. Overexpression of p59*fyn* in thymocytes yields hyperstimulable cells, supporting the hypothesis that p59*fynT* plays a critical role in T cell signalling (Cooke et al.,1991). To further define the role of p59*fyn* we have disrupted the thymic isoform of *fyn* in embryonic stem cells. The disruption vector used contained 7.5 kb of genomic *fyn* sequence with the *neo* gene inserted into exon 7B and with the HSV-TK gene attached to the 3' end of the vector. After positive/negative selection, 1 in 7 gancyclovir-G418 resistant clones contained a targeted allele. Chimeric mice generated from two independently derived ES cell clones have transmitted the mutated allele through the germline and heterozygotes are now being bred to homozygosity. These animals will provide a useful substrate for the analysis of regions that define the specificity of p59*fyn* function.

M 102 PROTEIN KINASE C EXPRESSION IN B6SUtA HEMATOPOIETIC CELLS.

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Protein kinase C (PKC) has been implicated in the signal transduction pathway of both interleukin 3 (IL-3) and erythropoietin (EPO). With the availability of the B6SUtA cell line, a hematopoietic cell line which requires IL-3 for proliferation but which also responds to EPO, we have further characterized protein kinase C expression in response to EPO by means of the immunoblotting technique. Previous experiments in our laboratory using immunocytochemistry revealed immunostaining of PKC in the nuclei of B6SUtA cells treated with EPO. Although both beta I and II isoforms for PKC were identified the beta II appeared to predominate. By means of immunoblots we have also substantiated a nuclear localization of PKC as detected with commercially available polyclonal antibodies. Nuclei prepared from cells pre-treated with EPO appeared to display a more prominent band at approximately 80 kD than did cytosol preparations when visualized on immunoblots. These data support our hypothesis that PKC in the nucleus may play an important role in regulating gene expression in hematopoietic progenitor cells.

M 103 THE 9A5 ANTIGEN IDENTIFIES EARLY MYELOID, T AND B CELL LINEAGES AND A SUBPOPULATION OF MATURE $CD4^+$ T CELLS.

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A monoclonal antibody (9A5) produced against mouse fetal thymocytes identifies an alloantigen on thymocytes, a subpopulation of T cells, and a minor population of bone marrow cells which have multipotent differentiation potential. The 9A5 mAB identifies a trypsin sensitive alloantigen on >80% of fetal thymocytes. The frequency of positive thymocytes and the intensity of their expression of this antigen decrease as a function of age. The $9A5^+$ thymocytes include both $CD3^-$ and $CD3^{lo}$ cells, many of which express both CD4 and CD8. In spleen and lymph nodes, 9A5 is selectively expressed by a subpopulation of $CD4^+$ T cells. In bone marrow, the antibody identifies approximately 10% of the non-adherent mononuclear cells that are lymphoid in appearance, but are negative for B220, CD3, and μ chains. The $9A5^+$ cells in bone marrow are also negative for the BP-2 granulocyte marker but 50% of them co-express the Mac-1 antigen. To test the differentiation potential of this bone marrow subpopulation, sorted $9A5^+$ cells were (1) injected into SCID mice, (2) tested for CFU-S potential, (3) and cultured on the S17 stromal cell line. The results in SCID mice indicate that $9A5^+$ bone marrow cells can give rise to T lineage cells. The $9A5^+$ subpopulation is also enriched for CFU-S and, when cultured on S17 stromal cells in the presence of IL-7, gives rise to B lineage cells which express the B220 and BP-1/6C3 B cell antigens. The 9A5 antigen therefore appears to be a marker for precursors of myeloid, T and B cell lineages that is retained or re-expressed by subpopulations of thymocytes and peripheral $CD4^+$ T cells. (Supported by NIH grants AI30879 and CA13148, MDC is a HHMI investigator)

M 104 **Study of Tissue- and Stage-Specific Variable Region Gene (VDJ) Assembly Using Chimeric Mice Generated from Embryonic Stem (ES) Cells,** Jianzhu Chen and Frederick W. Alt, Department of Genetics, Harvard Medical School, Boston, MA 02115

We have specifically adapted and developed a novel approach to study tissue- and stage-specificity of V(D)J recombination by using chimeric mice generated from ES cells. In this approach, recombination substrates are introduced into ES cells. ES cells harboring the substrates are injected into mouse blastocytes to generate chimeric mice. DNA is isolated from different tissues or different stages of B and T cells and the VDJ recombination status of the substrates is determined. By varying DNA elements in recombination substrates, cis-DNA elements which regulate tissue- and stage-specific VDJ recombination can be delineated. This approach allows a more physiological assay than previously used methods that involve cell lines. Furthermore, it allows pre-selection of copy number of the constructs and is faster and less expensive than the transgenic method because establishing mouse lines are not required. The approach could also be used to study tissue-specific gene expression and function in general.

Our basic recombination substrate contains an unrearranged TCR VDJ gene segment and the constant region gene segment of μ heavy chain. This construct, which does not contain any known transcriptional enhancer, is not rearranged in chimeric mice as detected by both genomic Sothern and PCR. When the intron Eμ enhancer is inserted between unrearranged VDJ and Cμ segments in the basic construct the substrate is rearranged for DJ in both B and T cells and for VDJ predominantly in T cells. Thus, the Eμ enhancer clearly affects tissue-specific VDJ recombination. When the intron Ek enhancer is inserted between unrearranged VDJ and Cμ segments, the substrate is not rearranged. Currently, we are investigating what specific DNA motif(s) in the Eμ enhancer regulates tissue-specific recombination and why the Eκ intron enhancer does not have a similar effect.

M 105 DIFFERENTIATION OF LYMPHOID AND MYELOID LINEAGES FROM MOUSE EMBRYONIC STEM CELLS IN VITRO, Una Chen, Basel Institute for Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland

Mouse embryonic stem (ES) cells have the potential to differentiate into embryoid bodies in vitro mimicking normal embryonic development. This system might offer unique opportunities for the study of lymphohemopoietic differentiation, if it were possible to define culture conditions that allowed differentiation of ES cells into mature cells of different lineages. I report the findings concerning the establishment of several ES cell-derived lines that contain precursors for both lymphoid and myeloid lineages. These lines were created by immortalization of ES cells found in embryoid bodies 8-14 days after differentiation. The cells in these cultures roughly correspond to those found in yolk sac or fetal liver. For this purpose various combinations of retroviruses carrying different oncogenes (eg. v-abl, v-raf, c-myc), lymphokines (IL-2, IL-3 and IL7) and Concanavalin A were used. Using these techniques cell lines that could be categorized into seven groups according to their characteristics were established. By surface-immunofluorescence, cells of lymphoid and myeloid lineages were shown to have developed. Adoptive transfer of *these cells* into sublethally irradiated mice resulted in their differentiation into mature T and B cells including Ig secreting plasma cells. Thus, these cell lines contain immortalized precursors of all major lymphohemopoietic lineages. It is now possible to further dissect the molecular and cellular steps leading to lympho- and myelopoietic differentiation by closer defining stem cells or their committed progenitors found in these lines. In addition, this study might provide an alternative approach to look at embryonic differentiation and to specifically target genes into *cells of predetermined lineage.*

M 106 CHRONIC MYELOPROLIFERATIVE DISEASE INDUCED BY *SITE-SPECIFIC INTEGRATION OF* A-MuLV INFECTED HEMOPOIETIC STEM CELLS. Chung SW, Wong PMC, Han X. Morse Institute of Molecular Genetics, Department of Microbiology & Immunology, Box 44, 450 Clarkson Avenue, SUNY Health Science Center at Brooklyn, Brooklyn, New York 11203.

We recently showed that hemopoietic stem cells expressing the v-abl oncogene can cause leukemia when injected into lethally irradiated recipient mice (PNAS 88:1585, 1991). Progenitor cells expressing v-abl did not make a significant contribution to disease development and the leukemia was monoclonal in origin. By serially transplanting v-abl transduced hemopoietic stem cells into normal, non-irradiated syngeneic recipients, we now showed that multiple stem cell clones do exist in some recipients. They fluctuated as normal stem cells do, and could home to normal bone marrow. Based on the time course of disease, the recipients developed either an acute or a chronic phase of disorder. All recipients with the acute disease had stem cell clones with random A-MuLV integration sites. All recipients with the chronic disorder had a unique A-MuLV integration site. We hypothesize that this abl specific integration (ASI) site is associated with either the activation or inactivation of a gene product. Consequently, the expression of v-abl is suppressed or the signal transduction induced by v-abl is interrupted, giving rise to a modified leukemic developemnt. Thus this ASI locus plays an important role in abl-mediated stem cell leukemognesis.

M 107 *IN VITRO* EFFECTS OF TOXICANT-INDUCED OXIDATIVE STRESS ON *MURINE BONE MARROW CELL GM-CSF-INDUCED PROLIFERATION,* AND GRANULOCYTE/MACROPHAGE-COLONY FORMATION. Robert J. Colinas and David A. Lawrence. Dept of Microbiology, Immunology, & Molecular Genetics, Albany Medical College, Albany, NY 12208.

Exposure to benzene (Bz), a major industrial pollutant, is linked to multiple hematopoietic disorders. Bz-induced hematotoxicity is believed to be mediated by one or more of its metabolites. Likely hematotoxic candidates are the thiol-reactive Bz metabolites hydroquinone (HQ) and 1,4-benzoquinone (BQ). *HQ and BQ may exert their toxic effects on hematopoietic progenitors* by inducing oxidative stress within the bone marrow (BM) as a result of thiol depletion. Continuous *in vitro* exposure of B6C3F1 murine BM cells for 24hr to 25μM HQ or BQ lowers total cellular thiols per unit protein by 20 and 55%, respectively. Treatment of BM cells with GM-CSF alone increases total thiols by 15%, perhaps reflecting GM-CSF-induced stimulation of the granulocyte/ macrophage (GM) lineages. Interestingly, addition of GM-CSF with HQ completely offsets HQ-mediated thiol modulation while GM-CSF *only partially* offsets the effects of BQ on BM cell thiols. In the absence of GM-CSF, HQ- and BQ-mediated cytotoxicity correlated closely with thiol depletion. However, GM-CSF alone exhibited slight cytotoxicity (6%). Therefore, it was not possible to determine whether the increased BM cell thiol content, induced by GM-CSF, protected cells from the effects of HQ or BQ. Results from continuous exposure of murine BM cells to 1 to 50μM concentrations of either HQ or BQ in GM-colony forming unit (CFU) assays showed that HQ has an IC_{50} of 20±5μM while the IC_{50} of BQ is 15±5μM. HQ inhibited GM-CSF-induced BM cell DNA synthesis, *which reflects the effects on the proliferative state of both* GM progenitors and the larger population of more mature myeloid cells, with a dose response curve similar to that obtained in the GM-CFU assay. In contrast, BQ inhibited BM cell proliferation >95% at ≥15μM. In addition, the extent of BQ cytotoxicity under the GM-CFU assay conditions was determined by flow cytometry using propidium iodide to stain nonviable cells. BQ cytotoxicity closely paralleled the effects of BQ on GM-CSF-induced BM cell proliferation. These results suggest that murine GM hematopoietic progenitors *are more resistant to BQ than the majority of BM cells. These results are* consistent with a recently published report suggesting that hematopoietic progenitors express significant levels of the multidrug efflux pump, P-170 (Cell 66:85-94, 1991). Therefore, it is possible that resistance of GM progenitors to BQ is due to P-170-mediated BQ efflux. Data from additional experiments delineating the role of the P-170 multidrug efflux pump in the resistance of GM progenitors to BQ will be presented.

M 108 THE BIOLOGICAL PROPERTIES OF PURIFIED MURINE MGF RECEPTOR NEGATIVE AND POSITIVE STEM CELLS, Peter de Vries, Ken A. Brasel, Hilary J. McKenna, Fred A. Fletcher and Doug E. Williams, Department of Experimental Hematology, Immunex Corporation, 51 University St., Seattle, WA 98101

Murine hematopoietic stem cells were purified using a widely applicable multiparameter flow cytometric procedure. Using biotinylated MGF, stem cells could be separated into c-kit^{neg} and c-kit^{pos} subpopulations. Day-14 CFU-S, and most types of in vitro colony forming stem cells, were highly enriched and almost exclusively found in the c-kit^{pos} fraction (10,747 ± 1,029 day-14 CFU-S per 10^5 injected cells). In contrast, the c-kit^{neg} fraction contained roughly the same number of day-14 CFU-S as unseparated bone marrow.

In vitro culture of both stem cell fractions resulted in a 400-fold and 40-fold net production of day-14 CFU-S in the c-kit^{neg} fraction and c-kit^{pos} fraction, respectively. Cells in both fractions are able to reconstitute the lymphoid compartments in SCID mice, the thymus of sublethally irradiated normal mice, and cells in the spleen, thymus, blood and bone marrow of lethally irradiated recipients in a competitive long term reconstitution assay.

Based on the kinetics of reconstitution observed, we postulate that the c-kit^{neg} stem cells are more primitive than the c-kit^{pos} stem cells which contain all the colony forming stem cells.

M 109 A *DROSOPHILA* SYSTEM FOR STUDYING THE REGULATION OF HEMATOCYTE STEM CELL PROLIFERATION AND DIFFERENTIATION. Charles R. Dearolf[1], Richard Binari[2], Norbert Perrimon[2], and William Hanratty[3], [1]Dana Farber Cancer Institute and Joint Center for Radiation Therapy, Harvard Medical School, Boston MA, 02115; [2]Dept. of Genetics, Harvard Medical School, Boston, MA 02115; [3]Dept. of Biology, Univ. of So. Mississippi, Hattiesburg, Miss. 39406.

The fruit fly *Drosophila melanogaster* offers powerful genetic and molecular techniques to systematically characterize regulatory pathways which control developmental processes such as hematopoiesis. The *Tumorous-lethal* (*Tum-l*) mutation causes an overproliferation and premature differentiation of fly hematocyte stem cells, resulting in melanotic masses, invasiveness into other tissues, and lethality. We have found that the *Tum-l* mutation is in a locus which encodes an apparent protein tyrosine kinase. We are now conducting mutagenesis screens to identify other genes whose products interact with *Tum-l* in the same signal transduction pathway. In addition, we are developing self-renewing tissue culture lines to provide a large-scale source of fly hematocyte stem cells.

M 110 INVOLVEMENT OF A DIVERGED HUMAN HOMEOBOX GENE, HB24, IN HEMATOPOIESIS AND LYMPHOCYTE ACTIVATION. Y. Deguchi, G.L. Wilson, and J.H. Kehrl. Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892

Proteins encoded by homeobox containing genes are sequence-specific DNA-binding proteins which have been implicated in the control of gene transcription both in developing and adult tissues. A recently characterized human homeobox gene, HB24, has been found to be expressed in hematopoietic progenitor cells and activated lymphocytes. When the homeodomain from HB24 was compared to known mammalian and Drosophila homeodomains it was found to be only moderately conserved (35-48%), intermediate between the prototypic HOX families and the highly diverged POU-domain-containing proteins. CD34 positive but not CD34 negative cells express HB24 and treatment with IL-3 and GM-CSF in vitro resulted in a rapid increase in HB24 mRNA transcripts (within 1 day) followed by a gradual decrease (essentially absent by day 7). However, HB24 can be re-induced in lymphocytes, following lymphocyte activation there is an increase in HB24 mRNA transcripts. To examine the effects of HB24 expression in T cells, HB24 was stably transfected into Jurkat cells under the control of a constitutive promoter. The high level expression of HB24 conferred a phenotype suggestive of activated T cells. A number of genes known to be induced following T cell activation and associated with T cell growth are increased in the transfectants. Besides the phenotype of activated T cells the HB24 Jurkat cells proliferated more rapidly than control cells and the increase in proliferation was directly related to the presence of HB24. These findings implicate HB24 as an important regulatory gene in both the growth and differentiation of hematopoietic progenitor cells and in activated lymphocytes.

M 111 THYMUS REPOPULATING CELLS ARE PRESENT IN THE UNANCHORED FRACTION OF STEROID-FREE BONE MARROW CULTURES, Marie-Ange Deugnier, Monique Denoyelle, Zita Prakapas, Catherine Dargemont and Jean Paul Thiery. CNRS, Ecole Normale Supérieure, 75005 Paris, France.

In an attempt to enrich and characterize rat T-cell precursors resident in bone marrow, we have developed stroma-dependent cultures of bone marrow precursor cells. In 3-day cultures grown on bone marrow feeder cells under steroid-free conditions, the lymphoid cell compartment composes 30% of all non-adherent cells and the frequencies of precursor cells with CFU-S and CFU-GM activity are respectively 5- and 15-fold reduced as compared to day 0. At day 3, 85% of the lymphoid cell compartment is composed of rat pre-B cells (HIS24$^+$ HIS50$^+$ cells) and 15% are more phenotypically immature lymphoid cells (HIS24$^+$ HIS50$^-$ cells and HIS24$^-$ HIS50$^-$ cells). Using a rat thymopoiesis assay in fetal thymic organ culture (1), we found that the non-adherent cultured cell population taken at day 3 is up to 10-fold enriched in thymus repopulating activity, as compared to fresh bone marrow cells at day 0. Intrathymically, both fresh and cultured cells give rise to predominantly Thy-1.1^{high} CD5^{low} CD2$^+$CD3$^-$ TcR$\alpha\beta^-$ CD4$^-$ CD8$^-$ immature thymocytes and also to CD4$^-$ CD8$^+$ TcR$\alpha\beta^{-/low}$ and CD4$^+$ CD8$^+$ TcR$\alpha\beta^{-/low}$ cells. In the bone marrow cultures grown under steroid-free conditions, thymus repopulating activity is associated with HIS24$^-$ HIS50$^-$ cells and with HIS24$^+$ HIS50$^-$ cells but not with HIS24$^+$ HIS50$^+$ cells. These cultured precursor cells show a reduced intrathymic proliferative potential as compared to fresh bone marrow. Similar cultures maintained in the presence of 10^{-7} M hydrocortisone are strongly depleted in non-adherent lymphoid precursor cells able to repopulate thymic lobes in vitro. These data show that T-cell precursors can be separated from early myeloid precursors while being enriched in the unanchored cell population of stroma-dependent bone marrow cultures grown under conditions favoring B- lymphopoiesis.Such cells could fulfill criteria of committed T-cell precursors released from the bone marrow.

(1) Deugnier et al. (1990) Eur. J. Immunol. 20, 2075-2081.

M 112 ISOLATION AND CONCENTRATION OF HEMATOPOIETIC PROGENITOR CELLS (HPC) FROM HUMAN UMBILICAL CORD BLOOD, Kimberly P. Dunsmore, Alice Stewart and Joanne Kurtzberg, Pediatric Bone Marrow Transplant Laboratory, Duke University Medical Center, Durham, NC 27710
Human umbilical cord blood (UCB) contains increased numbers of HPC as compared to human bone marrow (vol:vol) and has recently been successfully utilized as an allogeneic source of hematopoietic stem cells to reconstitute bone marrow after lethal myeloablative therapy (NEJM 321: 1174-78, 1989). It has been proposed that UCB may be utilized as a source of banked autologous or allogeneic cells for bone marrow transplantation. Toward this end, we examined two methods of isolation of mononuclear (MN) cells from UCB which could be used to minimize the volume and red blood cell content of the cryopreserved sample. UCB was obtained from term infants at delivery and HPC were asssayed by quantitating the numbers of CFU-GM, CFU-GEMM and BFU-E and determining cell surface antigen expression of the immature antigens CD7 and CD34 on unprocessed samples and on mononuclear cell fractions obtained by either density gradient centrifugation or automated processing with an albumin/saline gradient on a Baxter Fenwal CS3000 Plus. Characteristics of MN cell populations recovered by these two methods are shown below.

	MN	CD34	CD7	PROGENITORS GM	GEMM	BFUE
	(% of unprocessed sample)					
DENSITY GRADIENT	88	70	174	123	160	94
AUTOMATED CS3000 PLUS	70	185	106	40	20	63

If UCB can serve as a useful source of hematopoietic stem cells, concentration of MN cells and elimination of red blood cell content will allow for more efficient cryopreservation and for increased availability to a larger donor pool. Our studies show that this can be accomplished without significant loss of HPC. Characterization of HPC in UCB will contribute to improving methods of lymphohematopoietic stem cell isolation and transplantation with human cord blood.

M 113 c-kit-POSITIVE CELLS IN THE HUMAN BONE MARROW Hideo Ema[1,2], Robert E. Donahue[1] and Neal S. Young[1]
1. ClinicalHematology Branch, NHLBI, National Institutes of Health, Bethesda, MD 20892, 2. Department of Molecular Biology, Princeton University, Princeton, NJ 08544
Stem cell factor (SCF) has been identified as the ligand for tyrosine kinase receptor encoded by c-kit proto-oncogene. SCF/c-kit has been implicated as playing important roles in hematopoiesis and stem cell migration. In order to study the differentiation potential of c-kit-positive cells in the human bone marrow, we analyzed bone marrow cells stained with anti-c-kit product (SR-1) and CD34 by FACS. Sorted c-kit-positive and/or CD34-positive cells were cultured in the presence of hematopoietic growth factors including SCF. c-kit was expressed in 7.6% of the bone marow cells and 3.1% of the total cells in the blast gate set. CD34 antigen was expressed in 7.8% and 3.2% *of these cells. In CD34-positive cells, 62% of the cells co-expressed* c-kit product. We sorted (1)CD34-negative,c-kit-positive cells, (2)CD34-positive,c-kit-positive cells and (3)CD34-positive,c-kit-negative cells individually from bone marrow using the blast gate and then incubated 1,000 cells each in a methylcellulose system. In CD34-negative,c-kit-positive cells, CFU-e, BFU-e and CFU-G/GM accounted for 18%, 5% and 3% of the total cells. In CD34-positive cells, 98 % of the total colony-forming cells were present in c-kit-co-expressing cells. In suspension culture of c-kit-positive cells with SCF, c-kit product disappeared from almost all cells by day 6 along with the loss of CD34 antigen. c-kit is expressed in a wide range of hematopoietic progenitors in the human bone marrow.

M 114 IDENTIFICATION OF A PUTATIVE T CELL-SPECIFIC ENHANCER WITHIN THE LAST INTRON OF THE HUMAN CD8α GENE, John E. Hambor[#,*], Paula Kavathas[#] and Jeffrey H. Hanke[*], *Department of Molecular Genetics and Protein Chemistry, Central Research, Pfizer Inc., Groton, CT 06340, and #Departments of Laboratory Medicine, Genetics and Immunobiology, Yale University, New Haven, CT 06504.

The CD8α gene is exclusively expressed by a subset of T cells and NK cells. As an initial step towards understanding the molecular basis for tissue-specific expression of the human CD8α gene, we surveyed the surrounding chromatin structure for potential *cis*-acting regulatory elements by DNAse I hypersensitivity mapping. Analysis of a panel of $CD8^+$ and $CD8^-$ T cell lines, as well as non-T cell lines, revealed five hypersensitive sites. A 1.2kb DNA fragment, containing a prominent T cell-specific DNAse I hypersensitive site identified in the last intron, was subcloned upstream of a minimal HSV TK promoter/CAT reporter cassette. Transient transfection of this plasmid demonstrated enhanced activity in T cell lines, but not in non-T cell lines. An homology search identified a striking cluster of DNA consensus sequences for four lymphoid-specific transcription factors (TCF-1, GATA-3, LyF-1 and Ets-1) within a 600 bp region of this fragment. DNA mobility shift assays using a DNA probe containing the consensus sequence for the CD8α TCF-1 element revealed a retarded band representing a DNA binding activity present only in nuclear extracts from T cell lines. These results indicate that the human CD8α gene may be regulated, in part, by a putative T cell-specific enhancer located within the last intron.

M 115 TGF-ß1 AND Rb GENES CONTROL A COMMON PATHWAY TO MAINTAIN EARLY HUMAN HEMATOPOIETIC PROGENITORS IN Go PHASE. Jacques Hatzfeld, Ma-Lin Li, Eugene L. Brown, Hemchand Sookdeo, Jean-Pierre Levesque, Angelo Cardoso, Timothy O'Toole, Steven C. Clark and Antoinette Hatzfeld, Laboratoire C.N.R.S. de Biologie Cellulaire et Moleculaire des Facteurs de Croissance, I.C.I.G., Hopital Paul Brousse, 94804-Villejuif Cedex, France and Genetics Institute Inc., Cambridge, Mass. 02140, USA.

Various progenitor assays have been developed in vivo and in vitro to study the hematopoietic stem cell compartment. We have used antisense oligonucleotides to study the roles of transforming growth factor ß1 (TGF-ß1) and the retinoblastoma susceptibility (Rb) antioncogene in the negative regulation of proliferation of early hematopoietic cells in culture. The antisense TGF-ß1 sequence significantly enhanced the frequency of colony formation by multi-lineage, early erythroid and granulo-monocytic progenitors but did not affect colony formation by late progenitors. These results can be observed both in serum or serum-free culture conditions. Single cell culture and limiting dilution analysis indicated that autocrine TGF-ß1 is produced by a subpopulation of early progenitors. Antisense Rb yielded similar results in releasing multipotential progenitors (CFU-GEMM) from quiescence. Rb antisense could partially reverse the inhibitory effect of exogenous TGF-ß1. Anti TGF-ß1 blocking antibodies, antisense TGF-ß1 or Rb oligonucleotides all had similar effects. No additive effects were observed when these reagents were combined, suggesting a common pathway of action. Our results are consistent with the model that autocrine production of TGF-ß1 negatively regulates the cycling status of early hematopoietic progenitors through interaction with the RB gene product. We will show how these results allow us to compare early progenitors/various CD34+ subpopulations/detected in different stem cell assays.

M 116 DEVELOPMENTAL POTENTIAL OF HEMATOPOIETIC STEM CELLS EXPRESSING RETROVIRAL IL-6, Robert G. Hawley, Andrew Z.C. Fong, Bruce F. Burns* and Teresa S. Hawley, Division of Cancer Research, Sunnybrook Health Science Centre and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M4N 3M5 Canada, and *Department of Anatomical Pathology, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9 Canada
IL-6 is a pleiotropic cytokine that participates in immune, inflammatory and hematopoietic processes. Recent studies with transgenic mice or with mice whose hematopoietic systems were reconstituted with retrovirus-infected bone marrow cells have demonstrated that sustained hyperexpression of IL-6 can lead to a polyclonal plasmacytosis resembling Castleman's disease (1,2). Enhanced hematopoiesis has also been seen in mice with increased serum levels of IL-6 (2-4), consistent with the finding that IL-6 promotes the proliferation/differentiation of hematopoietic progenitor cells *in vitro*. Nevertheless, varying *in vivo* effects have been reported, presumably reflecting differences in the extent to which different target cell populations are stimulated. To further examine the ability of IL-6 to act at early stages of hematopoiesis, we have constructed a new retroviral vector, MSCV, based on the MESV vector of Grez and colleagues which functions in embryonic stem cells (5). Mice receiving bone marrow cells expressing the MSCV-IL6 virus present with a dramatic myeloproliferative disease (peripheral leukocyte counts greater than 100,000/µl) prior to death four to twelve weeks post-transplant. In view of the massive amplification of hematopoietic cells in these mice, we assessed the repopulating ability of MSCV-IL6-infected stem cells by serial bone marrow transplantation. Interestingly, successful transfer of the disease to secondary, tertiary, and even quaternary recipients has been possible. Southern blot analysis of proviral integration patterns in hematopoietic tissues of serially-reconstituted recipients is currently underway. Taken together with the hematologic data accrued, the lineage studies should provide a more detailed picture of the stem/progenitor cell activities of IL-6.

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M 117 PERIPHERAL BLOOD STEM CELL MOBILIZATION: RAPID ENRICHMENT OF PROGENITOR CELLS USING A UNIQUE BIOTIN-AVIDIN IMMUNOAFFINITY SEPARATION SYSTEM, Shelly Heimfeld, William Bensinger, Barbra Fogarty, Kirsten McGuire, Sheryl Williams, Krisztina Zsebo and Ronald Berenson, CellPro, Inc., Bothell, WA 98021, Amgen, Inc., Thousand Oaks, CA 91320 and Pediatric Oncology, Fred Hutchinson Cancer Research Center, Seattle, WA 98104
Treatment of patients with chemotherapy reagents and/or growth factors leads to significant increases of progenitor cells in the blood. We have analyzed this progenitor cell mobilization, comparing blood samples from baboons treated with recombinant human Stem Cell Factor (rhSCF), and from human patients given rhG-CSF. CD34+ cells rise from a base line of <0.05% to 1-2% of the total white cells after treatment with rhSCF, 0.2-0.5% following rhG-CSF. Methylcellulose cultures show a 10-500 fold increase in colony-forming cell (CFC) progenitors per ml of blood following either growth factor treatment. Differences in the types of CFC progenitors mobilized by rhSCF as compared to rhG-CSF will be presented. We have developed a unique biotin-avidin immunoadsorption system to provide rapid enrichment of CD34+ progenitor cells. Using this system, 10^9 peripheral blood white cells have been run on a small 1.5 ml research device, with a total processing time of 2 hours. By FACS analysis the enriched cells were between 50-90% CD34+. Colony assays indicate a 50-100 fold enrichment for CFC, with >50% recovery of the total CFC activity. There was a direct correlation between %CD34+ and the level of CFC in the enriched samples. The separation results were similar between the rhSCF and rhG-CSF samples. These results indicate that this biotin-avidin immunoaffinity system can be used to rapidly enrich for mobilized peripheral blood stem cells, which should proved useful both for basic research and for clinical transplantation.

M 118 MOUSE MRP8 AND MRP14, TWO INTRACELLULAR CALCIUM-BINDING PROTEINS ASSOCIATED WITH THE DEVELOPMENT OF THE MYELOID LINEAGE, Eric Lagasse and Irving L. Weissman. MRP8 and MRP14 are two S100-like calcium-binding proteins of unknown function, associated with numbers of human inflammatory disorders. Both molecules have been described as L1 complex, cystic fibrosis antigen or p8 and p14. Here we report the cloning of mouse MRP8 and MRP14 and their pattern of expression during hematopoiesis. Mouse MRP proteins are coexpressed in fetal myeloid progenitors, where they are detected as early as day 11 of gestation. In fetal liver and yolk sac, MRP+ cell populations increased in number, in association with the development of the myeloid lineage. In adult mouse we identified MRP8 and MRP14 proteins in immature myeloid cells of the bone marrow, myeloid cells in the splenic red pulp and marginal zone in addition to monocytes and blood neutrophils. However, MRP expression is lost as cells terminally differentiate into tissue macrophages. All these experiments indicate that MRP proteins may have an important function in myeloid lineages, being expressed during the differentiation of early myeloid progenitors to mature cells. They have a possible role as calcium-binding proteins in this process.

M 119 HEMATOPOIETIC GROWTH FACTORS STIMULATE HIGH LEVEL MULTI-LINEAGE ENGRAFTMENT OF HUMAN BONE MARROW TRANSPLANTED INTO IMMUNE-DEFICIENT MICE, Tsvee Lapidot, Francoise Pflumio, Monica Doedens, Barbara Murdoch, Douglas E. Williams* and John E. Dick. Dept of Genetics, Research Institute, Hospital for Sick Children and Dept. of Molecular and Medical Genetics, University of Toronto 555 University Ave, Toronto, Ontario, Canada M5G 1X8; Dept. of Exptl Hematology, Immunex Corp.*
A complete understanding of the organization of the human hematopoietic stem cell hierarchy and of the molecular events regulating the stem cell developmental program has been hampered by the absence of suitable in vivo stem cell assays. These events are better understood in the mouse where single stem cells can rescue a lethally irradiated mouse by repopulating its marrow with both myeloid and lymphoid lineages. Recent advances in the transplantation of human cells into immune-deficient mice provides an opportunity to study human hematopoiesis-both normal and abnormal-in the context of a small animal model.

Our in vivo model for human hematopoiesis was designed to reflect as close as possible the current approaches of human bone marrow transplantation using IV injection of adult human bone marrow into conditioned immune deficient mice. We have evidence that the murine microenvironment can support human stem cells, but extensive differentiation does not occur probably because of the abscence of human growth factors. Treatment of transplanted animals with human mast cell growth factor (ligand for *c-kit*) and/or PIXY321 (IL3-GMCSF fusion protein) dramatically enhances the level of engraftment. There was extensive repopulation of the mouse bone marrow with human cells of multiple lineages. In addition to commited progenitors of myeloid and erythroid lineages, multi potential progenitors (CFU-GEMM) were present that gave rise in vitro to multi lineage colonies composed of megakaryocytes, erythrocytes, eosinophils, macrophages and granulocytes. The mice also contained differentiated human lymphoid and myeloid cells, and mature red blood cells could be elicited by exogenous administration of EPO. Human IgG was detected in all mice, and some mice had both IgG and IgM. In some mice cytokine treatment was started one month post-transplantation, and again multi lineage engraftment of immature and mature human cells was observed providing evidence that human stem cells can engraft the mouse stroma. This system represents a novel in vivo assay for human stem cells and for growth factors that stimulate their developmental program and offers a new approach to establish animal models of human hematopoietic diseases.

M 120 MOBILIZATION OF PRIMITIVE AND LATE BLOOD STEM CELL SUBPOPULATIONS IN THE MOUSE WITH CYCLOPHOSPHAMIDE (CY) AND/OR G-CSF P. Mauch, and S. Neben. Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA.

Various subpopulations of hematopoietic stem cells serve different functions in re-establishing hematopoiesis after lethal irradiation and stem cell transplantation. Progenitor cells are important for early engraftment while primitive stem cells (pre-CFU-S) appear responsible for maintenance of long-term hematopoiesis. Peripheral blood stem cells (PBSC) from unstimulated mice are deficient in both progenitor cell and primitive stem cell content. To study techniques to mobilize both progenitor and primitive stem cells from the marrow to the blood, we collected heparinized peripheral blood from C57BL/6 mice 6 days after a single iv dose of 200 mg/kg CY, after rhG-CSF (250 ug/kg/day b.i.d. subcutaneously for 4 days), or after CY followed by rhG-CSF. Erythrocytes were removed by centrifugation through Ficol-Hypaque. Significant increases in blood counts (WBC x 10^6/ml) and in CFU-S content per 10^6 blood cells were seen with CY (WBC-30.4 x 10^6/ml; CFU-S-80.1/10^6 cells), rhG-CSF (44.1; 45.5), and CY + rhG-CSF (66; 75.5) compared to unstimulated control mice (21.7; 8.6). Blood stem cells were analyzed for primitive stem cell content by Rs, an assay for CFU-S self-renewal, and competitive repopulation index (CRI), an assay of long-term repopulating ability. Both unstimulated and mobilized blood cells were deficient in primitive stem cell content compared to normal marrow (Rs-23.2; CRI-0.43). However, the primitive stem cell content of blood stem cells was significantly increased with CY (Rs-2.9; CRI-0.21), rhG-CSF (5.5, 0.41), and CY + rhG-CSF (6.4, 0.28) compared to unstimulated mice (2.9, 0.006). Mobilization following CY + rhG-CSF was documented by a marked loss of CFU-S in the marrow, a large increase of CFU-S in both the blood and spleen, and a loss of CRI in the marrow. The CFU-S content of the blood, marrow, and spleen returned to normal by 30 days. These data show that CFU-S content per 10^6 cells in the blood reaches near normal marrow levels after mobilization, the mobilization from the marrow to the blood is temporary and reversible, primitive stem cells can be mobilized abeit at a lower level than committed progenitor cells, and that the specific technique used may mobilize different subpopulations of stem cells.

M 121 HEMATOPOIETIC DEVELOPMENT *IN VITRO* FROM EMBRYONIC STEM CELLS. Terrill K. McClanahan and Frank Lee. DNAX Research Institute, Palo Alto, CA 94304. Embryonic stem cells derived from mouse blastocysts will undergo a program of differentiation *in vitro* which resembles early stages of mouse embryonic development *in vivo*, including formation of hematopoietic cells in visible blood islands. We have analyzed this system to study the role of cytokines in the formation of hematopoietic stem cells. Using PCR analysis we found low level expression of many hematopoietic growth factors and their receptors in embryonic stem cells and temporal increases in expression of certain of these genes as *in vitro* development proceeds. Of particular interest is the c-fms gene whose expression increases dramatically as embryonic stem cells develop into embryoid bodies. This expression also correlates with the appearance of macrophages in *in vitro* derived embryoid bodies grown in methylcellulose cultures, as evidenced by hematological staining. We have also analyzed these developing cultures for the presence of lineage specific cell-surface markers by FACS staining. Our results indicate that some lineage markers such as F4/80, a macrophage marker, are increased on the cell surface as *in vitro* development proceeds. We have assessed the potential of embryonic stem cells to differentiate and form hematopoietic stem cells by utilizing *in vivo* assays including spleen colony formation and contribution to the hematopoietic lineages of a developing mouse embryo by *in utero* transplantation. Our preliminary evidence indicates that cells from *in vitro* derived embryoid bodies have some capacity to function as hematopoietic stem cells *in vivo*.

M 122 MVI VECTOR STRATEGY FOR DIFFERENTIAL MARKING OF HEMATOPOIETIC STEM CELLS
Kateri A. Moore, and John W. Belmont, Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston TX 77030. Retroviral vectors that uniquely mark cells are being used to study the relatedness of different stem cell pools and to optimize conditions for gene transfer into hematopoietic stem cells (HSC). A family of vectors, termed MVI for multiple vector insert, were generated by modifying the N2 vector with different sized inserts. The resultant proviruses are distinguishable by PCR amplification with one set of oligonucleotide primers. Ten different vectors were generated and are packaged by both ecotropic, GP+E-86, and amphotropic, GP+E-AM12, lines at high titer. A series of murine bone marrow transplantation experiments have been undertaken with five of these vectors to assess the relationship between primary CFU-S, MRA-CFU-S (marrow repopulating ability), LTCIC (long term culture initiating cell) and the repopulating HSC. Transduction was achieved by either co-cultivation with virus-producing cells or incubation with cell-free virions in the presence or absence of exogenous growth factors. The growth factors studied were IL-3, leukemia inhibitory factor (LIF) and mast cell growth factor (MGF) separately and combined. An initial experiment demonstrated a 77% infection efficiency for MRA-CFU-S derived from HSC enriched mouse BM infected by cell-free virions. We have also used two of these vectors as cell-free supernatants to differentially mark control and experimental cell populations of human bone marrow. The experimental conditions assessed the effects of exogenous growth factor stimulation and the effects of stromal cell monolayers on human stem cell transduction in competitive long-term cultures. The growth factor combinations tested were IL-3 plus Il-6, LIF, MGF, and LIF plus MGF. Pre-established irradiated monolayers from allogeneic bone marrow cells provided the stromal support. A transduction efficiency of 65% for clonogenic cells (CFC) from human LTCIC was observed with stromal support that was not replaced by exogenous growth factors.

M 123 MECHANISM OF PROTECTION OF HEMATOPOIETIC PROGENITORS FROM 4-HC AND IRRADIATION BY IL-1 AND TNFα., Jan Moreb, Julie A. Eastgate, James R. Zucali and Harry S. Nick, Departments of Medicine, Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610
Pretreatment with IL-1 and/or TNFα has been shown to protect hematopoietic progenitors both in vivo and in vitro from irradiation and chemotherapy. In this study, we have investigated the mechanisms of such protection. First, we studied the role of aldehyde dehydrogenase (ALDH), an enzyme responsible for the metabolism of 4-hydroperoxycyclophosphamide (4-HC), in the protection of human progenitors from 4-HC. Human marrow mononuclear cells were incubated for 20 hours with culture medium alone (control); IL-1 + TNFα; IL-1 + TNFα + cycloheximide; or IL-1 + TNFα with Diethylaminobenzaldehyde (DEAB) added for the final 10 minutes of incubation. All groups were treated for 30 minutes with a lethal dose of 4-HC. The cells were then washed and cultured using the blast cell colony assay. Colonies were scored weekly. Results showed significant protection of early progenitors by IL-1 + TNFα. This protection was prevented by cycloheximide and DEAB suggesting that protein synthesis and specifically ALDH synthesis were necessary for the protection. Next we studied the role of manganese superoxide dismutase (MnSOD) in the protection of murine progenitors from irradiation. Murine bone marrow cells were incubated with IL-1, TNFα or both IL-1 + TNFα versus medium alone (control) prior to irradiation at 800 rads and then placed in modified long-term Dexter cultures. In parallel, total RNA was isolated from similarly incubated cells, northern blotted, and then probed for MnSOD mRNA. The long-term culture results showed significant protection from irradiation by IL-1 and/or TNFα in comparison to control, while the northern analysis showed a significant increase in MnSOD mRNA in cells protected by IL-1 and/or TNFα. Mice pretreated with IL-1 prior to lethal irradiation were also studied. There was increased survival following irradiation and their bone marrow cells contained significantly greater levels of MnSOD mRNA when compared to media treated animals. These studies suggest that multiple mechanisms may be induced by IL-1 and/or TNFα in the protection of early hematopoietic progenitors from different therapies.

M 124 ISOLATION OF MOUSE ES-CELL DERIVED HEMOPOIETIC STEM CELLS

Albrecht M. Müller and Elaine Dzierzak, GSE, Mill Hill, The Ridgeway, LONDON, U.K.

The maintenance of stem cells is a strategy of organisms to generate and continously supply cells of the intestinal, epidermal and hemopoietic systems. The stem cells responsible for hemopoiesis in the adult reside in the bone marrow, while during embryonic and fetal stages, the cell(s) reside(s) in the yolk sac and liver, respectively. This poses the following questions (i) whether there is a direct lineage relationship between hemopoietic stem cells (HSC) active in different locations during the different developmental stages or whether they are distinct cells and (ii) which processes guide the initiation of the embryonic HSC. Because stem cells are rare, not well characterised and difficult to grow in vitro, alternative systems to obtain the difficult cells should be developed.

Examination of the embryonic origin of the hemopoietic stem cell of the mouse can take advantage of the totipotent embryonic stem cells (ES-cells) which have been shown to contribute to the germline even after in vitro culture for many generations (Evans and Kaufman, 1981, *Nature* 292: 154; Martin, 1981, *PNAS* 78: 7634). A prerequisite for our work is the demonstrated ability of ES-cells to differentiate in vitro into various hemopoietic lineages, such as erythroid, myeloid, granulocytic cells (Doetschman, et al., 1985, *J. Embryol. exp. Morph.* 87: 27; Wiles and Keller, 1991, *Dev.* 111: 259). ES-cells have also been demonstrated to provide an excellent in vitro system for the analysis of the erythropoietic differentiation program (Lindenbaum and Grosveld, 1990, *Genes & Dev.* 4: 2075). For these reasons we believe that ES-cells can be used to isolate hemopoietic stem cells.

To test for the in vivo hemopoietic repopulating ability of ES-cells, such cells are transferred into immunodeficient murine recipients after differentiation in vitro in the presence of hemopoietic growth factors. To optimise the number of transferred ES derived stem cells we enrich for hemopoietic stem cells by procedures described for adult bone marrow and fetal liver stem cells (Spangrude et al., 1988, *Science* 241, 58; Jordan et al., 1990, *Cell* 61: 953).

Preliminary data indicate that ES-cells may be used as an in vitro system for the isolation and characterisation of embryonic hemopoietic stem cells. The employment of the ES-cell system could be used to elucidate mechanisms during the earliest onset of in vivo hemopoiesis.

M 125 STEM CELL HETEROGENEITY REVEALED BY ANTIBODY FALL-3

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A major goal in the study of hematopoiesis is to characterize and isolate pluripotent stem cells. We have generated a panel of monoclonal antibodies that detect immature cells in murine bone marrow. One of these antibodies, Fall-3, detects primitive hematopoietic stem cells with extensive repopulation capacity. Immunofluorescence analysis showed that Fall-3 differs from a variety of previously described antigens that are expressed also on stem cells, including Sca-1. This suggests that Fall-3 defines a novel stem cell antigen. We have previously reported that Fall-3 allowed the separation of pluripotent stem cells from a developmentally early B lymphocyte precursor. Now, we have evidence that this antibody reveals heterogeneity in the stem cell compartment itself. When we separated bone marrow cells into Fall-3^{hi} and Fall-3^{lo} populations, we found that both populations contained radioprotecting cells and stem cells that reconstitute lethally irradiated mice for many months. However, in a competitive repopulation assay, only Fall-3^{hi} cells competed well. Furthermore, only Fall-3^{hi} cells had significant self-renewal capacity as assessed in the pre-CFU-S assay. This suggests that the Fall-3^{hi} and Fall-3^{lo} stem cells differ in their proliferative capacity as predicted by the generation-age hypothesis. These different stem cells can now be separated with antibody Fall-3 for further characterization.

M 126 EXCESS CFU-S PRODUCTION IN NORMAL MOUSE HEMATOPOIESIS

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Hematopoiesis is represented by a hierarchy of developmental steps ending, in the mouse, in a daily production of 2.6-3.8 x 10^8 functional cells per day. The majority of these developmental steps can now be studied by different assays, some of them allowing for relatively precise quantitative estimates. The spleen colony assay detects clonogenic cells (CFU-S) characterized by their capacity to give rise, on average, to 10^6 cells per colony. The CFU-S had been thought to be nonproliferating or very slowly proliferating cells. However, more detailed measurements revealed 10 to 40% of CFC-S to be engaged in DNA synthesis at any time. A total number of CFC-S, i.e. CFU-S numbers corrected for seeding efficiency of the assay (designated then as CFC-S), is about 500,000 in the whole hematopoietic tissue of the mouse. A daily cell output derived from these numbers is at least 1,000 times higher than what would be necessary to produce normal numbers of functional blood cells. Therefore, either only a small fraction of CFU-S proliferative capacity is used in the steady state hematopoiesis or only a small fraction of CFU-S produced is used for blood cell production. A combination of these two alternatives is also possible. In any case, a considerable functional reserve seems to exist at the CFU-S developmental level.

M 127 SELF RENEWAL IS NOT A PROPERTY OF REPOPULATING HEMOPOIETIC STEM CELLS (pre-CFU-S).

Walter R. **Paukovits**, Marie-Hélène **Moser**, Johanna B. **Paukovits**, Dept.Growth Regul,Inst.Tumor Biol-Cancer Res., Univ. Vienna, Vienna-1070, Austria

Three consecutive injections of ara-C in mice (300 mg/kg, 12 hrs interval) have the following consequences: **a)** extensive progenitor and precursor killing, resulting in neutropenia. **b)** compensatory proliferation of the CFU-S, which thereby also become subject to ara-C killing. **c)** application of the CFU-S and CFU-GM inhibiting peptide pEEDCK prevents CFU-S decimation and neutropenia (Paukovits&Moser,Blood 77:1313(1991).

We have now investigated the **pre-CFU-S** population under these conditions, in vivo by the repetitive transplantation assay (Ploemacher), and in vitro by serial long term culture (Eaves).

Results: a) nine hrs after the last ara-C injection CFU-S are reduced to 10% of normal, with 90% in S-phase (by suicide), pre-CFU-S are reduced to 30%, none of them in S-phase (same as in untreated mice). **b)** two months later WBC counts, BM-cellularity, CFU-GM and CFU-S numbers are back to normal, while pre-CFU-S numbers are reduced to 11.5 %. pEEDCK-treated mice have significantly higher values (53%), post-chemotherapy stimulator treated mice are at 11%. **c)** twelve months later pre-CFU-S are still at this level. **d)** in vitro long-term-culture-initiating cells show the same pattern.

We conclude: a) pre-CFU-S are an exhaustible storage population; **b)** few remaining pre-CFU-S suffice to generate an apparently normal hemopoietic activity, repopulation reserves may however be severely reduced. **c)** preserving CFU-S numbers by pEEDCK results in reduced pre-CFU-S consumption, improving the repopulation ability of the marrow; **d)** protection by inhibitors is superior to post-chemotherapy stimulation in avoiding long term damage; **e)** progression of pre-CFU-S into CFU-S does not involve proliferation; **f)** pre-CFU-S do not recover from drug induced decimation. High self-renewal activity would be expected under such conditions, indicating that **g)** self-renewal is not a property of hemopoietic stem cells.

M 128 EFFECTIVE ISOLATION OF HUMAN PROGENITOR CELLS; A NEW METHOD FOR DETACHMENT OF IMMUNOMAGNETIC BEADS FROM POSITIVELY SELECTED CD34+ CELLS,

Leiv Rusten[1],Torstein Egeland[3],Steinar Funderud[1],Heidi Kiil Blomhoff[1],Gunnar Kvalheim[2] and Erlend B. Smeland[1]. [1]Laboratory of Immunology and [2]Department of Tumor Biology, The Norwegian Radium Hospital, and [3]Institute Transplantation of Immunology, Rikshospitalet, Oslo, Norway.

Most excisting methods for positive selection of CD34+ cells are hampered with variable yield and/or moderate purity of the isolated cells. In positive selection of CD34+ cells using immunomagnetic beads, unspecific binding of beads to cells represents a significant problem. To reduce unspecific binding and to increase recovery, we have tested My 10, 12.8, QBEND-10 and BI-3C5 anti-CD34 mAbs for optimal coupling to paramagnetic beads M450 (Dynal). Especially, use of beads directly coated with BI-3C5, markedly reduced the percentage of contaminating cells. Moreover, we have recently developed a successful method for detachment of immunomagnetic beads from positively selected cells, using an anti-Fab antiserum [DetachAbead (Dynal)]. By this detachment technique, the cells are rendered unaltered with an intact antigenic profile. The final yield was on average 1,3% of bone marrow mononuclear cells (range 0,8% to 2,3%, n=10), which constitutes 40 to 70 % of expected yield, as estimated from the percentage of CD34+ rosetting cells. The purity is consistently high (>95% CD34+ cells of the isolated cells). Isolated cells are markedly enriched for CFU-GM cells, and respond to IL3, GM-CSF, MCGF (Immunex), G-CSF and/or TNF, alone or in combinations in liquid culture. Moreover, the cells are also markedly enriched for blast colony forming cells, and the CD34-population are depleted 80-90 % of cells capable of blast colony formation. We conclude that the isolated cells show a normal response in a variety of in vitro assays. The capacity of these cells to engraft in animal models is currently under investigation.

M 129 ISOLATION AND CHARACTERIZATION OF $CD34^+$ HEMATOPOIETIC STEM CELLS BY IMMUNOSELECTION.

Lisa Schain, Mark Harvey, Thomas B. Okarma, and Jane S. Lebkowski. Applied Immune Sciences, Inc. Menlo Park, CA 94025.

Enrichment of CD34+ hematopoietic cells was achieved by a two-step procedure utilizing a unique technology whereby proteins are covalently attached to an activated polystyrene surface. In this process, bone marrow mononuclear cells (BMMC) are first incubated on a device containing covalently immobilized soybean agglutinin (SBA) in order to deplete many differentiated cell types. The non-adherent, SBA- cells are then incubated on a second device containing the CD34 Antibody, ICH3. The nonadherent CD34- cells are removed, and the positively selected CD34+ cells are then physically detached from the device by agitation. The physical and functional characteristics of these cells are consistent with those of hematopoietic stem cells. The purified CD34+ cells are agranular cells of small to medium size which express the CD34 Ag. Subsets of these cells express the HLA-DR, CD38, CD33, and CD19 antigens. These cells are >90% viable and are enriched 10-50 fold in CFU-C as compared to BMMC's. Moreover, these purified progenitors produce 10-50 fold greater activity in long term bone marrow culture as compared to BMMC's. To test the capabilities of these devices in tumor purging, CD34+ cells were purified from BMMC's intentionally contaminated with five different radiolabeled tumor cell lines. In each case, a 2.5 to >3.0 log depletion of tumor cells was observed. This technology has now been scaled up to conveniently process bone marrow samples up to $2x10^{10}$ BMMC. The utility of these devices in collecting peripheral blood stem cells is now under investigation.

M 130 BCL-2 PROMOTES MATURATION AND INHIBITS MULTIPLE FORMS OF APOPTOSIS BUT NOT NEGATIVE SELECTION IN THYMOCYTES.

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The proto-oncogene, Bcl-2, an inner mitochondrial membrane protein, has been shown to inhibit apoptosis in certain cell lines. In the thymus, Bcl-2 is regionally localized to the mature T cells of the medulla. To assess the role of Bcl-2 in the programmed death of thymocytes, we generated transgenic mice that redirected Bcl-2 expression to cortical thymocytes. Bcl-2 protected immature $CD4^+8^+$ thymocytes from glucocorticoid, radiation, as well as anti-CD3 induced apoptosis. Moreover, Bcl-2 altered T cell maturation resulting in increased percentages of $CD3^{hi}$ and $CD4^-8^+$ thymocytes. Despite this, clonal deletion of T cells which expressed Vβ5, Vβ11, and Vβ17a T cell receptors, which recognize endogenous superantigens, still occurred. This transgenic model indicates that multiple death pathways operate within the thymus that can be distinguished by their dependence on Bcl-2.

M 131 DEVELOPMENT OF SWINE HEMATOPOIETIC PROGENITOR ASSAYS AND LONG-TERM CULTURE SYSTEM,

J. Graham Sharp[1], Jim A. Rogers[2], Joan W. Berman[2], David A. Crouse[1] and Douglas M. Smith[3], Depts. of [1]Anatomy and [3]Path/Micro, Univ. Neb. Med. Ctr., Omaha, NE 68198 and [2]Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

Present cancer therapy may involve the collection of hematopoietic tissues, administration of high-dose chemotherapy and subsequent hematopoietic rescue with the stored material. Pre-clinical studies of bone marrow or peripheral stem cell transplantation and associated cytokine applications can be greatly facilitated by the use of large animal models. Physiological, radiological and hematological similarities make swine an excellent model system. While it has been shown that hematopoietic transplants are possible in swine, a limitation to their wide-spread use in research has been the lack of well defined in vitro culture assays for the progenitors of hematopoietic progenitors. Using human and mouse colony stimulating factors, we have developed assays for swine myeloid hematopoietic progenitors. These include assays for m and gmCFU-C, an assay for the primitive high proliferative potential colony forming cell (HPP-CFC) and an assay for long-term culture initiating cells (LTC-IC) using a pre-established mouse stromal cell line as the supporting adherent population. Using this system, we find that the range of progenitors present in normal pig marrow aspirates to be within the ranges found for the progenitor equivilants found in mouse and human. In addition, we have developed two different swine long-term bone marrow culture systems using either a variation of the Dexter method of long-term culture initiation, or a mouse stromal cell line (MS-5) as the adherent stromal environment. Using the above progenitor assays and flow cytometry, we have detected both primitive and committed progenitors in both systems over a period of 3 months. The availability of these assays and culture systems should make the miniature swine a more attractive model for both basic research and the development of clinical transplantion stategies.
(Supported in part by the Neb. Dept. of Health, LB506)

M 132 THE EARLIEST T-LINEAGE POPULATION IN THE ADULT MOUSE THYMUS CONSISTS OF LYMPHOID-RESTRICTED PRECURSOR CELLS, Ken Shortman, Li Wu and Roland Scollay, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.
We have recently isolated from adult mouse thymus a minute subpopulation representing the earliest T-lineage cells, at a stage preceding the $CD4^-8^-$ T precursors. These early precursors resemble hematopoietic stem cells in surface phenotype, except that they express moderate levels of CD4 and express SCA-2. They have TCR β and γ genes, and IgH genes, in germline state. On intrathymic transfer they take a week longer to produce mature progeny than $CD4^-8^-$ precursors, but they give a much higher progeny yield. They serve as precursors of γδ as well as αβ T cells. This "low CD4 precursor" population has no CFU-s activity. On intravenous transfer to Ly 5-congenic recipients, progeny cells are found in all lymphoid organs. No granulocytes or macrophages are among the progeny. However, B cells (s-Ig^+, $B220^+$) as well as T cells are among the progeny in bone-marrow, spleen and lymph nodes; only T-cell progeny are found in the thymus. This suggests the earliest precursor cells in the thymus are no longer multipotent stem cells, but do retain a potential to develop into B cells, as well as αβ- and γδ-T cells.

M 133 PHENOTYPIC IDENTIFICATION OF MURINE BONE MARROW CELLS WITH THYMUS REPOPULATING ABILITY. Walentina A.T. Slieker, Johannes C.M. van der Loo, Marella F.T.R. de Bruijn, Miranda R.M. Baert, Pieter J.M. Leenen, Rob E. Ploemacher and Willem van Ewijk, Department of Immunology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.
Relatively little is known about the phenotype of the bone marrow prothymocyte. Identification of surface determinants present on these cells would greatly facilitate their isolation and the study of the earliest stages of T cell development.
Recently, we produced two new monoclonal antibodies, ER-MP12 and ER-MP20, each recognizing cell surface determinants present on subpopulations of murine bone marrow cells. Using 2 colour flowcytometric analysis 6 subpopulations can be identified. In order to investigate whether prothymocytes are present in one of these subpopulations, we sorted these subpopulations using the FACS. Subsequently, we transferred the cells either by intravenous or intrathymic injection into sublethally irradiated mice, congenic for both Thy-1 and Ly-5 antigens. Thymus repopulating ability of the 6 subpopulations was also determined in vitro using fetal thymus lobes depleted of their endogenous thymocytes by deoxyguanosine. In addition, the frequency and the percentage of hematopoietic stem cells in the bone marrow subpopulations were determined using a limiting dilution type LTBMC assay (Ploemacher et al., 1989).
Our results show that prothymocytes and hematopoietic stem cells are exclusively present in the 2 subpopulations which lack expression of the ER-MP20 antigen, but express the ER-MP12 surface marker at intermediate and high levels. Cells with the highest thymus repopulating ability are found in the subpopulation with the brightest ER-MP12 antigen expression. This subpopulation also contains the majority of the less primitive, day 12 CFU-S like cells, as measured in the LTBMC, while the more primitive hematopoietic precursors are found in both subpopulations.

M 134 ABSENCE OF THY-1.2 EXPRESSION ON A SUBPOPULATION OF MOUSE HEMATOPOIETIC STEM CELLS. Gerald J. Spangrude, Laboratory of Persistent Viral Diseases, NIAID, Rocky Mountain Labs, Hamilton, MT 59840
Mouse hematopoietic stem cells (HSC) can be identified and enriched from populations of normal bone marrow cells by immunofluorescent labeling of cell surface molecules followed by flow cytometric separation. The majority of HSC activity, as defined by long-term repopulation of irradiated animals, can be localized to a fraction of bone marrow cells which expresses the Ly6A/E (Sca-1) molecule and does not express any of a panel of lineage markers that identify lymphoid, myeloid, and erythroid cells. Expression of the Thy-1.1 determinant has also been shown to be a characteristic of HSC in both rat and mouse, although expression is high in rats and low in mice. Analysis of HSC activity in bone marrow of mouse strains expressing the Thy-1.1 allele indicated that the vast majority of HSC activity was included in the Thy-1-low population. In contrast, mouse strains expressing the Thy-1.2 allele contain in their bone marrow a significant percentage of HSC activity among Thy-1-negative cells. Similar to Thy-1.1 strains, however, most HSC activity is contained in the Sca-1-positive, Lineage-negative population of cells. Isolation of Sca-1-positive, Lineage-negative, Thy-1.2-negative cells reveals that this cell population is highly enriched for long-term repopulating potential and pre-CFU-S activity, but contains 5-fold less CFU-S activity than does the Sca-1-positive, Lineage-negative, Thy-1.2-low population. In addition to a high frequency of CFU-S (1 per 9 cells), the Sca-1-positive, Lineage-negative, Thy-1.2-low population contains long-term repopulating cells and pre-CFU-S activity. The extent to which these activities can be physically separated from each other by further fractionation may influence the development of new in vitro assays for HSC activity.

M 135 TRANSGENIC MOUSE STUDIES ON THE FUNCTION OF THE ONCOGENE *bcl*-2.
A. Strasser, A.W. Harris, M.L. Bath, S. Whittingham, J. Adams and S. Cory. The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Parkville, 3050, Australia
Deregulated expression of the putative oncogene *bcl*-2 due to chromosomal translocation to an immunoglobulin locus has strongly been implicated in the aetiology of follicular centre cell lymphoma and some cases of chronic lymphocytic leukemia. *bcl*-2 was shown to be the prototype of a new class of oncogene which promotes cell survival rather than proliferation. In order to study *bcl*-2 function *in vivo*, we generated transgenic mouse lines harboring a human *bcl*-2 cDNA linked to the mouse 5′ *Igh* enhancer [Eμ]. Most lines showed transgene expression and phenotypic abnormalities in the B cell compartment only. These included significantly increased numbers of small resting B cells and Ig secreting plasma cells. *bcl*-2 promoted extended survival *in vitro* of cells from all stages of B cell development. Immunized Eμ-*bcl*-2 mice displayed 3-5-fold higher and dramatically prolonged immune responses to SRBC and LPS. These mice did not usually develop follicular centre lymphoma. However, within the first year of life 60% of them became terminally ill with a systemic autoimmune disease characterised by severe immune complex glomerulonephritis and high titres of antinuclear autoantibodies.
bcl-2 oncogene impact was also studied in Eμ-*bcl*-2 mice expressing the transgene in cells of the T lineage. T cells from all stages of development showed remarkably sustained viability (even in the presence of lymphotoxic agents) and some spontaneous differentiation *in vitro*. Although total T cell numbers and the rate of thymic involution were unaltered, the response to immunization was enhanced. No T cells reactive with self-superantigens appeared in the lymph nodes, but an excess was found in the thymus. All these observations suggest that modulated *bcl*-2 expression is a determinant of life and death during normal lymphocyte development.
The oncogenic potential of *bcl*-2 was revealed in Eμ-*bcl-2/myc* doubly transgenic mice which developed tumors much faster than Eμ-*myc* mice. Interestingly, the phenotype and genotype of Eμ-*bcl-2/myc* tumors indicates that they are neoplastic representatives of normal lymphoid progenitor or stem cells. Under certain conditions of cocultivation with stromal cells we could induce their differentiation to pro-myelocytes and B lineage cells. We plan to use these tumors to identify novel lymphoid progenitor specific marker molecules, genes and growth factors.

M 136 ENRICHMENT AND CHARACTERIZATION OF MURINE HEMATOPOIETIC STEM CELLS WHICH EXPRESS C-KIT MOLECULE, T. Suda, S. Okada, Y. Miura, S-I. Nishikawa, H. Nakauchi and K. M. Zsebo. Department of Medicine, Jichi Medical School Tochigi-ken, 329-04, Kumamoto University Medical School, The Institute of Physical and Chemical Research (RIKEN) and AMGEN Inc.

The proto-oncogene c-kit encodes a transmembrane tyrosine kinase receptor for stem cell factor (SCF). The c-kit/SCF signal is expected to have an important role in hemopoiesis. A monoclonal antibody (ACK-2) against murine c-kit molecule was prepared. Flow cytometric analysis revealed that the approximately 5% of bone marrow cells which expressed c-kit molecule were B220(B)$^-$, TER119(erythroid)$^-$, Thy$^{negative-low}$ and WGA$^+$. A small number of Mac-1(macrophage)$^+$ or Gr-1(granulocyte)$^+$ cells were c-kit-low positive. In vitro colony forming cells (CFU-C) and day 8 and day 12 CFU-S existed exclusively in the c-kit-positive fraction. About 20% of Lin(Lineage)$^-$c-kit$^+$ cells were Rhodamine-123^{low} and this fraction contained more day 12 CFU-S than day 8 CFU-S.

On the basis of these findings, murine hemopoietic stem cells were enriched from normal bone marrow cells. One out of 2 and 4 Thy-1^{low}Lin$^-$ WGA$^+$c-kit$^+$ cells were CFU-C and CFU-S, respectively. Long-term repopulating ability was investigated using B6/Ly5 congenic mice. Eight and 25 weeks after transplantation of Lin$^-$c-kit$^+$ cells, donor-derived cells were found in the bone marrow, spleen, thymus and peripheral blood. In peripheral blood, T cells (Thy1$^+$), B cells and granulocytes-macrophages were derived from donor cells. Moreover, Lin$^-$c-kit$^+$Sca-1$^+$ fraction (0.04% of total bone marrow cells) contained day 12 CFU-S and cells capable of reconstituting hemopoiesis in irradiated mice. A half of these cells responded to SCF + IL-3/IL-6 and formed in vitro colonies.

Injection of ACK-2 into the irradiated mice after bone marrow transplantation decreased the numbers of day 8 and day 12 CFU-S in a dose-dependent manner. Day 8 spleen colony formation was completely suppressed by the injection of 1 µg ACK-2, but a small number of day 12-colonies were spared. Our data show that c-kit molecule is expressed in primitive stem cells and plays an essential role in the early stage of hematopoiesis.

M 137 THE IN VITRO RESPONSE OF PLURIPOTENT HUMAN STEM CELLS AS DEFINED BY A CD34+THY1+LIN- POPULATION LuAnn Thompson-Snipes, Nancy Mori and Charles M. Baum. SyStemix, Inc., Palo Alto, CA 94303.

We have studied the growth factor requirements of a population of candidate human hematopoietic stem cells isolated from fetal bone marrow and purified by fluorescent cell sorting. These cells were selected for expression of CD34 and Thy 1 and lack of myeloid or lymphoid cell surface markers (Lin). Purified recombinant factors IL-1, IL-3, IL-6 and c-kit ligand were incubated alone or in combination with the isolated cells in a semi-solid media. After two weeks, colonies were identified by morphology and counted. In a comparison of CD34+Thy1+Lin-, (Lin-), and CD34+Thy1+Lin+, (Lin+), we found that the Lin- population demonstrated more BFU-e but fewer CFU-GM colonies than the Lin+ cells when incubated with IL-3 alone. The most striking difference, however, between these two populations of cells was in their response to a combination of two cytokines, c-kit ligand and IL-3. This combination of cytokines stimulated Lin- cells to form five times more colonies than Lin+ cells. Morphologically, most of the colonies derived from the Lin- cells were large and erythroid mixed (CFU-GEM). Thus, it appears that the CD34+Thy1+Lin- population has multipotential activity and is sensitive to c-kit ligand.

M 138 GENE EXPRESSION IN SINGLE REED-STERNBERG CELLS OF HODGKIN'S DISEASE: PCR GENERATED SINGLE CELL cDNA LIBRARIES Lorenz H. Trümper, Gerard Brady, Stefano Vicini, Jeff Cossman, Tak W. Mak, Ontario Cancer Institute, Toronto, Ontario M4X 1K9 and Georgetown University Medical School, Washington, D.C. 20007

The cellular origin of the Reed Sternberg (RS) cells, the presumably malignant cells of Hodgkin's disease (HD), still remains an enigma. Since these cells typically occur in low numbers in affected lymph nodes, they should ideally be studied at the single cell level. We have studied RS cells from lymphnodes of 7 patients obtained at diagnosis. RS cells were micromanipulated from single cell suspensions into a lysis/1st strand buffer for cDNA synthesis and subsequent homopolymer tailing. cDNA was sequence independent amplified an shown to be β-Actin positive from 64/98 cells picked. Approx. 10 RS cells from each case were examined. cDNAs were analyzed by probing with a panel of 3'UTR cDNA probes. Amplified cDNAs from cell lines served as controls for specificity of probing. Almost all single RS cells positive for β -Actin were positive for the proto-oncogene c-fes/fps. Also, c-myc and fyn gene expression was found in most RS cells. RS cells from 2/7 cases were positive for the src-family tyrosine kinase hck, which was not expressed in any of the RS cells from the other cases. RS cells from another two cases had frequent expression of lck and TCRβ chain. Expression of hck and lck was mutually exclusive. Expression of EBNA-1, EBNA-2 and LMP of EBV was not seen in RS cells, but found in EBV+ control lines. Expression of CD 19, CD 20, and CD 2 cDNAs was found in approx 20% of RS cells, with coexpression of T-and B-cell specific mRNA-transcripts in some cells. However, we could not determine if these transcripts were sterile, since the cDNA is approx. 600 bp long (from the polyA tail). Thus, we find heterogeneity of tyrosine-kinease gene expression at the single cell level between cases with consistency within cases. This might represent biological heterogeneity between different cases of HD. Expression of cytokines and their receptors will be studied. Single cell cDNA libraries will be constructed for subtractive analysis.

M 139 *In vivo* clonal analysis of mouse purified hematopoietic stem cells, Nobuko Uchida and Irving L. Weissman, Howard Huges Medical Institute, Department of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305

Mouse hematopoietic stem cells can be purified according to surface phenotype: Thy-1^{lo} Lin$^-$ Sca-1$^+$. Recently we have demonstrated that Thy-1.1^{lo} Lin$^-$ Sca-1$^+$ cells comprise the only adult C57BL/Ka-Thy-1.1 bone marrow subset that contains hematopoietic stem cells. In this study, limiting numbers (5-20 cells) of Ly-5 marked stem cells were transplanted into Ly-5 congenic, lethally irradiated host along with 2 x 10^5 host bone marrow cells. Donor-derived B and T lymphocytes, monocytes and granulocytes were traced in the blood over a period of 7 months. Two distinct outcomes were observed when donor-derived cells could be detected in the blood; 1) multi-lineage differentiation with transient myelopoiesis (short-term repopulation); or 2) multi-lineage repopulation with sustained myelopoiesis (long-term repopulation).

The order of appearance of donor-derived cells in the blood was myeloid (3-4 wk), B cells (4-5 wk) then T cells (6-8 wk). We never observed single-lineage restricted hematopoietic repopulation *in vivo*, such as B lineage-restricted or granulocyte lineage-restricted from Thy-1^{lo} Lin$^-$ Sca-1$^+$ stem cells. In conclusion, individual stem cells enter hematopoiesis *in vivo* uncommitted (=multi-potential).

M 140 EVIDENCE OF LONG-TERM REPOPULATING CAPABILITY OF AN IMMORTALIZED HEMOPOIETIC STEM CELL LINE. Wong PMC, Han X, Chen H & Chung SW. Morse Institute of Molecular Genetics, Dept. of Microbiology & Immunology, Box 44, 450 Clarkson Avenue, SUNY Health Science Center, Brooklyn, New York 11203.

We recently obtained an immature blast cell line, BL3, which carried a rearranged N2-IL3 retroviral genome that we used as a genetic tag for these cells. Immunofluoresence studies showed that these cells were Thy1+, Sca1+, B220-, and Mac1-, characteristic markers of hemopoietic stem cells. They could not directly develop CFU-S but could instead give rise to CFU-S forming cells. As few as 100 BL3 cells could be used to reconstitute lethally irradiated recipients. These animals were apparently normal at seven months after reconstitution, and DNA extracted from various organs, from lymphoid and myeloid cells freshly separated over a percoll gradient, and from spleen cells stimulated with either PHA or WEHI-3-conditioned medium, were shown to contain the BL3-specific markers. Approximately 10% of bone marrow and 60% of spleen hemopoietic colony forming cells were positive for the BL3 specific markers in recipient mice. Bone marrow cells from one recipient seven months after reconstitution were engrafted into secondary irradiated recipients, and 2-3 months later, BL3-specific markers were again shown to be present in DNA from organs of these secondary recipients. We also noticed the presence of a 3kb novel mRNA that hybridized specifically with the BL3 marker. We constructed an Okyama-Berg cDNA expression library from RNA of BL3 cells and were able to obtain one clone that contained a 3kb insert hybridized positively with the probe. We conclude that BL3 cells are hemopoietic stem cells with long-term repopulating capability. The putative cDNA we isolated may the the gene accounting for stimulation of hemopoietic stem cells to proliferate.

T Lymphocytes; Myeloid Growth Factors

M 200 NEONATAL AND ADULT T CELLS SHOW QUANTITATIVELY AND QUALITATIVELY DIFFERENT RESPONSES TO TCR STIMULATION, Becky Adkins, Kara Hamilton, and Grace Cross, Department of Pathology, University of Miami Medical School, Miami, FL 33136

Compared to adult animals, neonatal mice are immunodeficient. Many of their diminished immune responses in vivo are thought to be dependent on T cell activity. To investigate the mechanism(s) underlying these deficiencies, we have examined the responsiveness of neonatal and adult T cells to TCR-dependent and -independent stimuli in vitro. When stimulated with soluble anti-CD3 antibodies in the presence of adult accessory cells, neonatal T cells incorporate significantly less ^{3}H-TdR than do adult T cells. This reduction is seen over a wide range of anti-CD3 antibody concentrations and at both 24 and 48 hours of stimulation. Reduced proliferation is linked to both quantitative and qualitative differences in lymphokine production. Neonatal T cells produce less IL2 than do adult T cells at both 24 and 48 hours of stimulation. While adult T cells secrete little IL4 at either time point, neonatal T cells make almost exclusively IL4 by 48 hours of stimulation. The reduction in proliferation appears to be specific for stimulation via the TCR since neonatal T cells proliferate as well as adult T cells (although at different optimal concentrations of stimulants) in the presence of phorbol ester and calcium ionophore. We are currently investigating whether neonatal and adult T cells differ in (a) the frequencies of IL2 and/or IL4 secreting cells and (b) signal transduction events in response to TCR stimulation.

M 201 IDENTIFICATION AND CHARACTERIZATION OF A HIGH-AFFINITY GM-CSF RECEPTOR ON PRIMARY RAT OLIGODENDROYCYTES, Gayle Cocita Baldwin[1], Etty N. Benveniste[2], David W. Golde[3] and Judith C. Gasson[4], Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles CA[1]; Department of Neurology, University of Alabama, Birmingham AL[2], Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY[3]; Departments of Medicine (Hematology-Oncology) and Biological Chemistry, UCLA School of Medicine, Los Angeles CA[4]

Hematopoietic growth factor receptors are present on non-hematopoietic normal and neoplastic cells. We previously demonstrated GM-CSF receptors on tissues and cell lines that are derived from the neural crest. Recently, others have shown that another hematopoietic growth factor receptor, c-*kit*, is also expressed on neural crest-derived melanocytes, and that it is essential for the development of those cells, as well as development of primordial germ cells of the erythroid and mast cell lineages. As we are interested in normal non-hematopoietic cells that express GM-CSF receptors and may also be GM-CSF-responsive, we isolated and studied primary rat brain cells, including microglia, astrocytes and oligodendrocytes. Scatchard analysis of equilibrium binding of ^{125}I-GM-CSF to primary rat oligodendroyctes revealed 1,110 binding sites per cell, with a Kd of 20 pM. In six separate experiments, no specific binding was detectable on the astrocyte population. Microglia were used in competitive binding experiments with the oligodendrocytes, and addition of microglia did not increase the specific binding of oligodendroyctes to labeled ligand. In dose-response assays, we measured ^{3}H-thymidine uptake in oligodendrocytes, microglia and control murine 32D cells stimulated with various concentrations of GM-CSF. Over a concentration range of 0.025-1000 pM, we observed proliferation and peak ^{3}H-thymidine incorporation at approximately 30 pM for both the control cells and the oligodendrocytes. The microglia, however, did not proliferate in response to GM-CSF. These data indicate the presence of a functional receptor for GM-CSF on primary rat nerve cells, and suggest that hematopoietic growth factors may play a role in nerve cell development.

M 202 IL-10 INDUCES DIFFERENTIATION OF B LYMPHOCYTES INTO PLASMA CELLS.

Jacques Banchereau[1], Nadia Vezzio[1], Simone Peyrol[2], Jean-Alexis Grimaud[2], Françoise Rousset[1]
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When assessed over a 10-day period, IL-10 can enhance the proliferation of B cells incubated with both the mouse fibroblastic Ltk⁻ cell line that had been transfected with the human Fc receptor (FcγRII/CDw32) and monoclonal antibody to CD40. However, after two weeks, cells stopped proliferating and displayed an increased size. The cells grown in IL-10 gradually lost their B cell specific surface markers such as HLA class II antigens, CD19, CD20. Cells displayed intracytoplasmic Ig and secreted large amounts of IgM, IgA and IgG. Electron microscopy showed that these cells displayed an excentrated nucleus, a well developed cytoplasm with dense rough endoplasmic reticulum and Russel bodies. These features are consistent with a plasma cell morphology and indicate that IL-10 may be a cytokine inducing plasma cell differentiation.

M 203 *Abstract Withdrawn*

M 204 DEVELOPMENTAL ACQUISITION OF T CELLS IN HUMANS: IDENTIFICATION OF A SUBPOPULATION OF ACTIVATED T CELLS IN FETUSES.

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Most mature thymocytes express high levels of the RA isoform of CD45 (220 kD) but not the RO isoform (180 kD) and they exit the thymus with this naive T cell phenotype. Activation in the periphery results in loss of RA and upregulation of the RO isoform, the characteristic phenotype of memory T cells. Thus, in newborn blood and neonatal spleen, the RO^+ subpopulation of T cells is relatively small (<12%) but gradually increases to constitute approximately 50% of the circulating T cell pool in adults. When T cells in the fetal spleen (14-22 weeks gestation) were examined, to our surprise we observed that they resembled those of adults more than newborns in that up to 45% were RO^+. High levels of RO^+ T cells were also found in cord blood samples from premature but not term babies. RO^+ T cells the in fetal spleen were polyclonal in that they expressed a variety of V_β region determinants and were present in both CD4 and CD8 subpopulations. A subset of T cells expressing high levels of IL-2R (p55) were found in fetal and not adult spleens suggesting an ongoing immune response. The IL-$2R^+$ cells were almost exclusively $CD4^+RO^+$ and were responsive to IL-2. However, the $CD4^+RO^+$ cells from most fetal spleens failed to proliferate in response to anti-CD3 antibodies or to a combination of anti-CD2 antibodies which regularly induce proliferation of $CD4^+RO^+$ T cells from adults. The results suggest that fetuses possess a population of T cells that exhibit the typical surface phenotype of activated or memory T cells but lack the characteristic response pattern of immunologically mature T cells. (NIH grants AI 30879, CA 13148, AI 27290).

M 205 ABERRANT *Pim*-1 EXPRESSION LEADS TO ALTERED GROWTH FACTOR RESPONSES.

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The mouse *Pim*-1 gene encodes two cytoplasmic serine-threonine specific protein kinases. The highest *Pim*-1 mRNA expression is found in haematopoietic tissues like thymus and spleen, but also in gonads and ES cells. It is induced by growth factors like IL-2, IL-3 and GM-CSF. *Pim*-1 can be activated by MoMuLV in T and B cell lymphomas. Eμ-*Pim*-1 transgenic mice display a low incidence of T cell lymphomas and an increased susceptibility to carcinogens. Inactivation of the *Pim*-1 gene in mice following homologous recombination in ES cells yielded viable, fertile mice with no obvious phenotype. We analyzed the involvement of *Pim*-1 in growth factor signal transduction by investigating specific growth factor responses of cells with aberrant *Pim*-1 expression *in vitro*. The IL-3 response was investigated in Bone Marrow derived Mast Cells (BMMC). We found that IL-3 induced proliferation of BMMC lacking *Pim*-1 was severely impaired when compared to wildtype BMMC. However, the cells remained IL-3 dependend. Interestingly, apoptosis induced by growth factor deprivation was delayed. Overexpression of *Pim*-1 does not lead to IL-3 independence. The proliferative response of BMMC lacking *Pim*-1 to IL-4, IL-9 and MGF (steel) was normal. This shows that there was no overall reduction in proliferative capacity of these cell and that *Pim*-1 functions as an effector of the IL-3 response. In view of the involvement of *Pim*-1 in lymphomagenesis in mice we also started to look at growth factor responses in lymphoid cells. B-lymphoid cells derived from Whitlock-Witte cultures from Eμ-*Pim*-1 transgenic or control mice were subcultured in the presence of growth factor containing supernatants. Clones of fast growing cells with a primitive lymphoid phenotype ($B220^+$, sIg^-, $CD4^+$) were obtained from cultures containing the Eμ-*Pim*-1 transgene. We are currently investigating the growth factor requirements and differentiation capabilities of these cells.

M 206 INTERLEUKIN-11 IN COMBINATION WITH OTHER HEMATOPOIETIC GROWTH FACTORS SUPPORTS THE SURVIVAL AND EXPANSION OF PRIMITIVE MURINE PROGENITOR CELLS *IN VITRO*. Debra Donaldson, Steven Neben and Katherine Turner, Genetics Institute, Cambridge, MA 02140
Interleukin-11 (IL-11) is a cytokine that influences the growth, differentiation, and function of a number of cell types including hematopoietic progenitors, hepatocytes, preadipocytes, and lymphoid cells. In order to evaluate its effects on primitive hematopoietic progenitors 2-day post 5-fluorouracil bone marrow was incubated in suspension in vitro for six days with the addition of growth factors singly and in various combinations. The cultured cells were then evaluated for high proliferative potential colony-forming cell (HPP-CFC) and cobblestone area forming cell (CAFC) frequency, and in some cases *in vivo* competitive repopulating ability. IL-11, Steel Factor (SF), IL-3, IL-6, or G-CSF alone produced neither an expansion of committed progenitor cells nor survival of primitive stem cells (day 28 CAFC) in suspension culture. IL-11 produced approximately a 4-fold increase in HPP-CFC in combination with IL-3 and greater than 10-fold increase with SF. No synergistic activity was noted with IL-6. The 4-factor combination of IL-11, SF, IL-3, and IL-6 resulted in a 1-2 x 10^3-fold expansion of mature stem cells (day 7 CAFC) compared to a 50-100-fold expansion with SF, IL-3 and IL-6. Four-factor and 3-factor combinations were similar in allowing recovery of about 20-30% of input long term competitive repopulating ability. These results suggest that IL-11 has significant synergistic activity for amplification of progenitors without adversely affecting the viability of more primitive stem cells.

M 207 EFFECT OF IL-3 ON DIFFERENTIATION AND GROWTH OF THYMOCYTES AND SPLENIC T AND B LYMPHOCYTES IN IRRADIATED MICE, Gino Doria, Giorgio Leter, Marcello Spano' and Daniela Frasca, Laboratory of Immunology, AMB-BIO-MOL, ENEA C.R.E. Casaccia, Roma, Italy.
Interleukin 3 (IL-3) is a T cell-derived colony-stimulating factor that regulates hemopoiesis as it induces differentiation of pluripotent stem cells to mature cells of several lineages. Administration of murine recombinant (mu r) IL-3 to sublethally irradiated mice induced in the thymus complete recovery of the cell count and mitotic responsiveness to Con A. These results are consistent with the possibility that IL-3 either potentiates stem cell migration to the thymus or induces stem cell differentiation within the thymus. The latter possibility is sustained by the results of thymocyte fluorimetric analysis showing that injection of mu r IL-3 into irradiated mice induced a reduction in the percent of CD4-CD8- cells, which increases after irradiation, and full recovery of the CD4+CD8- cells. These findings strongly suggest that IL-3 induces the differentiation of immature T cells and selectively stimulates the proliferation of mature CD4+CD8- cells. In the spleen, the cell count and mitotic responsiveness to Con A and LPS as well as the antibody response and T helper cell activity were completely recovered by IL-3 treatment. The findings altogether show that IL-3 induces differentiation and growth of thymocytes and full recovery of splenic T and B cell functions in sublethally irradiated mice.

M 208 FUNCTIONAL STUDIES OF *HOX11*, THE HOMEOBOX GENE INVOLVED IN THE (10;14) TRANSLOCATION OF T-CELL NEOPLASIAS, Ian D. Dubé, Lilly Noble, and Suzanne Kamel-Reid, Oncology Research Program, Toronto General Hospital, and The University of Toronto Hospitals' Cancer Cytogenetics and Molecular Oncology Program, 100 College Street, Toronto, Ontario, Canada M5G 1L3.
We recently described the cloning of a novel human gene involved in the t(10;14) translocation of human T-cell leukemia *(Blood, 1991)*. The gene, *HOX11*, is transcribed into a 2.1kb transcript that encodes a 405 amino acid protein. The *HOX11* protein contains a helix-turn-helix motif that is highly conserved among members of the homeobox gene family and is most likely a DNA binding-domain. *HOX11* expression is deregulated in human leukemias with the acquired translocation t(10;14). As a part of our ongoing studies aimed at determining the role of *HOX11* expression in the pathogenesis of T-cell neoplasia, we subcloned the 2.1kb *HOX11* cDNA into an expression vector and co-transfected the plasmid with pSV2neo into NIH3T3 cells. G418 resistant clones were obtained that expressed *HOX11* transcripts. These clones were evaluated for their tumorigenicity in in vitro assays. Several clones exhibited altered growth kinetics and abnormal patterns of growth. They formed multiple foci within 3 weeks. When high-expressing *HOX11*/NIH3T3 clones were injected into immune-deficient mice they proliferated and metastasized. Our data suggest that constitutive expression of *HOX11* results in many features associated with transformation and supports the notion that deregulated expression of this gene is important in the pathogenesis of a subset of human leukemias. These finding will be presented and discussed.

M 209 IN VIVO ENRICHMENT OF T-PRECURSOR CELLS BY THY1-TK MEDIATED CONDITIONAL ABLATION, **E. Dzierzak, A. Muller, P. Fraser, B. Daly and C. Miles, National Institute for Medical Research, Mill Hill,The Ridgeway, London, England**
It has been suggested that the earliest T-precursor cell may be very closely related to the hemopoietic stem cell. Surface markers common to these two cell types have been demonstrated through FACS analysis and repopulation of deficient recipient mice. Thy1 is a surface determinant common to both cell types. To establish the lineage relationships between these cells we are utilizing a strategy wherely we can conditionally ablate the cells in vivo during ontogeny. We have produced mice transgenic for the Thy1 regulatory elements linked to the HSV-TK gene. In three lines of transgenic mice we have be able to eliminate >90% of the Thy1 expressing cells of the thymus through in vivo Gancyclovir treatment. The most efficiently ablated cells are those of the double positive CD4 and CD8 phenotype. Also, single positives and some double negative cells are ablated. Interestingly the remaining cells in the thymus are an enriched population of double negative cells, some but not all expressing IL-2 receptor. They express Thy1 at lower levels and are large, blast-like cells. Expression of HSA decreases slightly. Most interestingly, 5-6% of the cell express the fetal hemopoietic stem cell marker, AA4.1 and express CD4 to a low level. This is consistent with a cell type intermediate to the hemopoietic stem cell and the double negative thymic precursor cell. Thus, Thy1-TK induced ablation enriches for Thy1 low early thymic precursor cells of both CD4 low and double negative phenotype. We are testing for the functional repopulating ability of these cells.

M 210 THE EFFECT OF IL-3, IL-1 AND GM-CSF ON BLOOD ISLAND CELLS FROM CHICKVITELLINE MEMBRANE: Patricia Ferdinand, Ann Brown, Rene Laidlaw, Norma Brown, and Christopher George. Department of Natural Sciences and Mathematics, CUNY, Medgar Evers College, Brooklyn, N.Y. 11225.

Our aim was to develop a serum-free, EPO-free, newly developed organ culture system, to study the effects of IL-3, IL-1 and GM-CSF on erythroid progenitors from the blood islands of chick vitelline membrane. Small pieces of vitelline membrane from 3-4 day-old embryos in serum-free Iscove's medium were incubated at 37oC in a humidified 5% CO_2 /air incubator. After 4 days in culture, we observed mixed and typical CFUe colonies in vitro. Addition of IL-3 alone (1U/ml) and 2% FCS alone stimulated the increased production of colonies above control ($p < 0.001$). Addition of GM-CSF (1U/ml) alone and IL-1(1U/ml) alone inhibited the production of colonies compared to controls and cultures with 2% FCS ($p < 0.01$). Addition of IL-1 to IL-3 in culture did not inhibit the stimulatory effects of IL-3($p < 0.001$). Combination of IL-3 with GM-CSF did not override the inhibitory effects of GM-CSF alone ($p > 0.90$). *It is evident that chick* vitelline membrane blood island cells are responsive to hematopoietic regulators (IL-3,IL-1,and GM-CSF) that are effective in the mammalian system.

M 211 BIOLOGIC EFFECTS OF IL-10 ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS. Anne-Catherine Fluckiger, Françoise Rousset and Jacques Banchereau. *Schering-Plough, Laboratory for Immunological* Research, 27 chemin des peupliers, 69571 Dardilly, France

Our recent studies have established that IL-10 (viral and human) enhanced proliferation and allowed terminal differentiation of tonsillar B cells activated by anti-CD40 mAb presented on mouse Ltk⁻ cells stably expressing human *FcγRII/CDw32 (referred to as the CD40 system). Our present* study aimed at determining the ability of purified B-CLL cells to undergo proliferation and differentiation upon stimulation by IL-10, following cross-linking of either surface immunoglobulin (sIg) or CD40 molecule. Under activation by anti-Ig reagents (anti-IgM or Staphylococcus aureus particles -SAC-), we observed that: 1) B-CLL cells fail to proliferate in response to IL-10 and that IL-10 inhibits the DNA synthesis obtained in response to the activating agent in 8 out of 10 cases; 2) IL-10 inhibits the IL-2 induced proliferation of B-CLL cells activated through sIg. An opposite pattern of response is obtained in the CD40 system, since IL-10 potentiates the DNA synthesis of the great majority of B-CLL specimens and IL-10 synergizes with IL-2 to elicit B-CLL cell proliferation. IL-10 and IL-4 have additive effects on the proliferation of B-CLL cells activated by immobilized anti-CD40 mAb. The growth-promoting activity provided by IL-10 in the CD40 system, allows an important increase of viable cell numbers. In the CD40 system, IL-10 elicited B-CLL cells to produce IgM.

M 212 PARTIAL PURIFICATION OF BONE MARROW STROMAL CELLS IN CELLULAR AGGREGATES AND CHARACTERIZATION OF ASSOCIATED HEMOPOIETIC CELLS, Phillip E. Funk[1] and Pamela L. Witte[1,2], Departments of [1] Microbiology and [2]Cell Biology, Loyola University Medical Center, Maywood, IL 60153

Stromal cells form the hemopoietic microenvironment of the bone marrow. The lineage derivation, growth factor production, and location within the marrow of normal stromal cells is largely unknown. To address these issues we are attempting to isolate stromal cells directly from murine bone marrow. We have found that stromal cells are enriched in cellular aggregates that can be isolated from marrow cell suspensions by sedimentation. We show that aggregates seem to preserve areas of the native marrow architecture as staining for alkaline phosphatase and VCAM1 (detected by mAb M/K2) reveal a reticular pattern identical to that reported in marrow sections. Most culturable stromal cells are found within the parameters of high forward and right angle light scatter by FACS analysis. Within these parameters collagenase dispersed aggregates also display a definite increase in the frequency of M/K2⁺ cells when compared to whole marrow. This implies that stromal cells may be separated from other cells by sorting based on these parameters and M/K2 staining. Aggregates are also enriched in TdT⁺ cells as compared to whole marrow, suggesting that early lymphoid progenitors may be in contact with stromal cells *in vivo*. In addition, aggregate cells are more mitotically active than unfractioned marrow as assessed by thymidine incorporation, again suggesting that more primitive precursors are present within the aggregates. (Supported by American Cancer Society Grant IM-62877).

M 213 RECOMBINANT HUMAN INTERLEUKIN-11 (rhIL-11) STIMULATES MEGAKARYOCYTOPOIESIS AND INCREASES IN PERIPHERAL PLATELET NUMBER IN NORMAL AND SPLENECTOMIZED MICE. S. Goldman, T. Neben, J. Loebelenz, L. Hayes, K. McCarthy, J.B. Stoudemire, and R.G. Schaub, Division of Preclinical Biology, Genetics Institute, Cambridge, MA 02140

rhIL-11 is a recently cloned cytokine with biological activities affecting several hematopoietic cell lineages. *In vitro*, rhIL-11 synergizes with IL-3 to stimulate CFU-MEG. We have examined the effects of rhIL-11 on mouse megakaryocytopoeisis *in vivo*. Normal C57/bl mice and splenectomized C57/bl mice were treated for 7 days with 150 μg/kg rhIL-11 administered subcutaneously. In normal mice, peripheral platelets counts were 120% of vehicle injected controls after 3 days of rhIL-11 treatment. After 7 days of treatment with rhIL-11, platelet counts increased to 140% of controls. On day 10, 3 days after rhIL-11 dosing had ended, treated animals maintained platelet counts that were 120% of vehicle treated controls. Splenectomized mice treated with rhIL-11 showed platelet responses that were similar to normal treated mice during the first 7 days of the study. On day 10, however, the platelet counts in treated, splenectomized mice were no longer elevated. Analysis of bone marrow megakaryocyte ploidy by two color flow cytometry showed an increase, relative to controls, in the percentage of 32N megakaryocytes in both normal and splenectomized animals treated for three days with rhIL-11. A similar increase in the percentage of 32N megakaryocytes was seen on day 7 in both groups of rhIL-11 treated animals. On day 10, the bone marrow megakaryocyte ploidy distribution in rhIL-11 treated normal and splenectomized mice was the same as in vehicle treated controls. In normal mice, spleen MEG-CFCs were increased 2-3 fold relative to controls after 3 and 7 days of rhIL-11 treatment, while bone marrow MEG-CFCs were increased only on day 7. The number of MEG-CFCs in the bone marrow of rhIL-11 treated, splenectomized mice was increased 2 fold compared to controls on both days 3 and 7 of the study. These data show that treatment of normal or splenectomized mice with rhIL-11 can stimulate megakaryocyte progenitors, endoreplication of bone marrow megakaryocytes, and increased production of peripheral platelets. The responses of normal and splenectomized animals were similar although there were some kinetic differences. These studies suggest that clinical evaluation of rhIL-11 in the management of thrombocytopenia associated with disease states or marrow ablative therapies might be justified.

M 214 MOLECULAR REGULATION OF THE IL-3 GENE IN FRESHLY ISOLATED HUMAN LYMPHOCYTES. Lisa Gottschalk, Diane Giannola, Stephen Emerson, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109. The growth factor, IL-3, is a potent regulator of hematopoiesis. Produced exclusively by T and NK cells, IL-3 bridges the immune system and non-immune tissue homeostasis by influencing the development of both early stem cells and hematopoietic cells committed to distinct lineages. IL-3 is distinct from other growth factors by its restricted cellular expression and its obligate Ca^{+2} requirement for induction. While much is known regarding the biological activities and requirements for induction of IL-3 in vitro, relatively little is known concerning the regulation of expression of this gene. We performed Northern blot analysis on RNA isolated from peripheral blood lymphocytes (PBL) incubated with PMA and ionomycin and from human tumor cell lines (Jurkat, PEER) incubated with a variety of agents. We observed high level IL-3 expression in human PBL in contrast to extremely low level expression in the tumor cell lines. Since human tumor cell lines express low levels of IL-3 transcripts not comparable to levels expressed by normal human PBL and we have successfully transfected human PBL, we indirectly examined transcriptional regulation of the IL-3 gene by transient transfection of heterologous DNA constructs containing IL-3 genomic subfragments ligated to the bacterial CAT reporter gene and performed CAT assays. Similar to studies of IL-3 regulation in the non-human gibbon T cell line, MLA-144, 300bp of the IL-3 genomic 5' flanking region significantly increased CAT activity compared to that given by the CAT vector alone. However, a smaller subfragment containing 270bp of 5' flanking sequences and a silencer motif, NIP, was essentially inactive in stimulated human PBL. Further DNA sequence analysis of the IL-3 gene revealed motifs not previously observed including a sequence highly similar to NIP at -657 (8 of 10 bp), an *ets-1* nuclear protein binding site and the CD28 responsive element. Site directed mutagenesis and deletion analysis revealed the relative importance of each of the nuclear protein binding sites. For example, mutation of the NIP site in the -270 construct increased CAT activity by 48-fold in contrast to the 3-fold activity given by the wildtype -270 construct. Also, mutation of the *ets-1* and AP-1 nuclear protein binding sites in the -300 construct resulted in reduced CAT activity. Delineation of the cis-acting DNA sequences and the trans-acting nuclear DNA binding proteins should lead to understanding of the molecular basis for T cell specific, Ca^{+2} activation-dependent IL-3 gene induction and explicate the role of T cells in hematopoiesis.

M 215 HUMAN RECOMBINANT IL-10 IS AN INHIBITOR MOLECULE FOR ALLERGEN AND LECTIN DRIVEN T-CELL PROLIFERATION IN PBMNC CULTURE WITH LIMITED SPECIES CROSS-REACTIVITY, Michael J. Grace, Loretta A. Bober, Catherine C. Pugliese-Sivo, Faribourz Payvandi, Tracey A. Waters, Lee M. Sullivan and Satwant K. Narula, Biotechnology-Cell Biology, Schering-Plough Research, Bloomfield, NJ 07003.
Purified CHO-derived recombinant human IL-10 was found to inhibit allergen mediated T-cell proliferation in PBMNC cultures derived from atopic human donors. Maximal inhibition was achievable with as little as 10 U/ml of IL-10 in culture. The inhibition was abrogated by the addition of purified 19F1 monoclonal antibody against human IL-10. Two-color flow cytometry of the monocytes in PBMNC culture with IL-10 revealed a loss in surface Class II MHC (DR) and concomitant increase in surface CD14. Inhibition was also observed in Con A lectin driven PBMNC cultures taken from atopic human donors. Again, T-cell proliferation was blocked by IL-10, and the above respective changes in monocyte surface antigens observed. Human IL-10 inhibited to an extent lectin driven T-cell proliferation in PBMNC cultures taken from cynomolgus monkeys. However, purified murine IL-10 was not inhibitory to lectin driven T-cell proliferation in either human or cynomolgus PBMNC cultures. Further investigation is underway regarding human IL-10 effects on T-cells derived from lower species. We confirm that IL-10 may exert its regulatory role on the immune response by a T-cell suppressive effect. The molecule is not capable of broad species cross-reactivity using this system.

M 216 ANALYSIS OF HEMOPOIETIC CELLS IN *c-abl* GENE DISRUPTED MICE. Jeff Hardin[1], Sharon Boast[1], Pam Schwartzberg[1], Grace Lee[4], Fred Alt[1,2,4], Alan Stall[2], Elizabeth Robertson[3] and Stephen Goff[1,2]. Dept. of Biochemistry and Molecular Biophysics[1], Dept. of Microbiology[2], Dept. of Genetics and Development[3], and Howard Hughes Medical Institute[4], Columbia University, New York, NY 10032.

Mice homozygous for a mutation in the *c-abl* tyrosine kinase have multiple abnormalities including high post-natal mortality, runting, and susceptibility to pulmonary infections. Analysis of the immune system of homozygous mutants using Fluorescence Activated Cell Sorting (FACS) analysis has shown significant but variable reductions of B and T lymphocyte populations, the most severely affected being B cell precursors in the bone marrow. All other hemopoietic lineages appear normal in *c-abl* mutants.

Further characterization of the immune deficiency in the mutants *in vitro* using colony forming unit (cfu) assays of homozygous adults demonstrates a reduced response to cytokines in the same B cell populations observed to be reduced *in vivo*. Specifically, cfu-pre-B (IL-7 stimulated) in bone marrow and cfu-B (LPS stimulated) in bone marrow and spleen are reduced in homozygotes compared to littermate controls. Response to other cytokines (cfu-IL-3, cfu-GM, cfu-M, cfu-GEMM) appears normal.

Bone marrow transfers of *c-abl* mutant donors into lethally irradiated histocompatible hosts demonstrates that the mutant hemopoietic stem cells are capable of reconstituting hemopoiesis and the recipients do not appear susceptible to infections. However, by FACS analysis, about half of the animals receiving mutant bone marrow have reductions of B and T lymphocytes comparable to the reductions seen in mutant donors. Recipients of mutant fetal liver (donors were embryonic day 14 and day 19) do not have lymphocyte reductions. These results suggest that the *c-abl* mutation acts in a cell-autonomous manner in adult bone marrow and that the role of *c-abl* in adult bone marrow and fetal liver is different.

M 217 A DEVELOPMENTAL SWITCH IN B LYMPHOPOIESIS, Richard R. Hardy and Kyoko Hayakawa, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

B and T lymphocytes are generated from hematopoietic stem cells during both fetal and adult life. A critical unresolved issue is whether the differentiation pathways in lymphopoiesis are the same in fetal and adult animals or whether they differ, similar to the hemoglobin switch in erythropoiesis. We report here that a developmental switch occurs in B lymphopoiesis. We isolated Pro B cells using multiparameter flow cytometry from fetal and adult sources and investigated their B cell progeny generated both *in vitro* and *in vivo*. Most of the B cells generated from fetal liver, but few from adult bone marrow, expressed CD5. Further, fetal Pro B cells failed to generate cells expressing high levels of IgD following transfer into SCID mice, whereas adult Pro B cells gave rise to $CD5^-$ B cells bearing IgD at levels comparable to the bulk of cells in the spleen of adult mice. Furthermore, we found that a specificity normally over-expressed in the $CD5^+$ B cell population (to a determinant on mouse erythrocytes revealed by treatment with the proteolytic enzyme bromelain) was similarly enriched in the fetal Pro B repopulated animals, but not in those that received adult Pro B. Thus, all committed B progenitors in fetal liver of day 16 gestation mice give rise to phenotypically and functionally distinct progeny when compared to cells at a comparable differentiation stage in the bone marrow of adult animals. We conclude that the cohort of B lineage progenitors in early fetal development is committed to a differentiation pathway distinct from that in the adult and whose selected progeny survives in the adult largely as $CD5^+$ B cells.

M 218 IDENTIFICATION OF DNASE I HYPERSENSITIVE SITES THAT FLANK THE CHICKEN IMMUNOGLOBULIN LAMBDA LOCUS, Elizabeth A. Hurley and Craig B. Thompson, Howard Hughes Medical Institute and Departments of Internal Medicine and Microbiology/Immunology, University of Michigan Medical Center, Ann Arbor, MI 48109.

The genetic composition of the chicken immunoglobulin light chain lambda locus (Igλ) and the mechanism of the generation of diversity within this locus are well characterized. The Igλ locus is composed of a single functional V element, Vλ1, which rearranges to single joining (Jλ) and constant (Cλ) region genes in B cells prior to their migration to the bursa of Fabricius. Located upstream of Vλ1 are 25 pseudo V genes, which are used to diversify the rearranged Igλ by a process of intrachromosomal gene conversion occurring in the bursa. The entire unrearranged chicken Igλ locus encompasses less than 30 kb of DNA.

It has been suggested that higher order chromatin structure may influence the processes of transcription, rearrangement and the generation of diversity of Ig genes. We have explored the chromatin structure of chicken Igλ by mapping DNAse I hypersensitive (hs) sites in lymphoid and non-lymphoid tissues. Lymphoid-specific hs sites are detected within both the rearranged and unrearranged Igλ alleles. Several hs sites specific for the rearranged allele occur in the vicinity of the rearranged VJ gene. These sites may reflect protein-DNA interactions involved in initiating gene conversion events.

The small size of the chicken Igλ locus also allowed us to map DNAse I hs sites that flank the entire locus. Three hs sites have been identified in the 5 kb of DNA 5' of the pseudo V gene cluster; one of these sites is bursal-specific, while two are present in both B and T cells. At the 3' end of the locus, two hs sites have been identified in the 4 kb downstream of Cλ. One of these is present in both B and T cells, and the other is B cell-specific. Isolation of DNAs containing these hs sites and functional studies are in progress to determine if hs sites flanking the chicken Igλ locus constitute a locus controlling region (LCR).

M 219 TUMOR NECROSIS FACTOR-α (TNF-α) DIRECTLY AND INDIRECTLY REGULATES HEMATOPOIETIC PROGENITOR CELL PROLIFERATION: ROLE OF CSF RECEPTOR MODULATION. [1]S.E.W. Jacobsen, [1]F.W. Ruscetti, and [2]J.R. Keller, [1]LMI-BRMP-, [2]BCDP-PRI/Dyncorp, NCI-FCRF, Frederick, MD.

TNF-α has been shown to both stimulate and inhibit the proliferation of hematopoietic progenitor cells in vitro. It is, however, not known whether the effects of TNF-α are direct or indirect, or by which mechanism(s) TNF-α exerts its effects. We have found that the direct effects of TNF-α on proliferation of highly enriched murine bone marrow progenitors are inhibitory, regardless of the CSF stimulating growth. These inhibitory effects are correlated to a rapid trans-down-modulation of CSF receptor expression. In contrast, TNF-α indirectly stimulates CSF-stimulated bone marrow colony formation through the induction of at least G-CSF and CSF-1. This enhancement is preceded by an upregulation of GM-CSF and IL-3 receptor expression observed by 24 hours, is indirect and mediated at least in part by G-CSF. The modulation of CSF receptors was specific since TNF-α did not affect the expression of a number of other cell surface proteins, as well as the expression of the c-kit receptor. Thus, CSF receptor modulation might be involved in the direct inhibitory, as well as the indirect stimulatory effects of TNF-α on hematopoietic progenitor cells.

M 220 SIGNAL TRANSDUCTION THROUGH LAMBDA5 COMPLEXES ON PRE-B CELLS, J.Jongstra, V.Misener and W.-M. Zhu, Department of Immunology, University of Toronto and the Toronto Western Hospital, Toronto, Ontario, Canada M5T 2S8.

Early mouse pre-B cell lines carry partially rearranged or unrearranged IgH genes and do not express intact IgM-H protein (mu-protein). Such early mu^- pre-B cells express a cell surface protein complex consisting of the pre-B cell specific 22 Kd protein lambda5 and a 16 Kd protein, p16. Preliminary evidence suggests that a 140 Kd protein (p140) is present as a third component of this complex. Late pre-B cell lines which express intact mu-protein, express a lambda5/p16/mu-protein complex on the surface. To investigate a possible signal transduction function of the lambda5/p16/p140 and lambda5/p16/mu complexes on the surface of pre-B cell lines we measured the changes in intracellular free Ca^{2+} after treatment of cells with anti-lambda5 or anti-mu antibodies. Early mu^- pre-B cells showed a rapid and transient increase in intracellular free Ca^{2+} when incubated with anti-lambda5 antibodies but not when incubated with anti-mu. This response appeared to be due to an influx of Ca^{2+} across the plasma membrane. Late mu^+ pre-B cell lines showed a similar increase in intracellular Ca^{2+} after incubation with anti-lambda5 or anti-mu. Other responses of pre-B cells to engagement of the lambda5 complexes are currently investigated. Thus using a series of pre-B cell lines we show the existence of specific cell surface lambda5 complexes in different stages of pre-B cell development and show that both complexes can transduce an external signal to the inside of the cell.

M 221 A LOW-AFFINITY GM-CSF/ERYTHROPOIETIN CHIMERIC RECEPTOR FUNCTIONS IN MURINE CELL LINES. P.T. Jubinsky,* D.G. Nathan, and C.A. Sieff. Division of Pediatric Hematology and Oncology, Children's Hospital and Dana-Farber Cancer Institute, and Department of Pediatrics, Harvard Medical School, Boston, MA.

In order to identify domains in hematopoietic growth factor receptors that are important for signal transduction, a chimeric receptor was constructed consisting of the extracellular and transmembrane domains of the human GM-CSF receptor α subunit and the cytoplasmic domain of the murine erythropoietin receptor. Several factor-responsive murine hematopoietic cell lines, including FDCP1, BA/F3, and DA3 were co-transfected with a neomycin-selectable marker and either the chimeric receptor or the native hGM-CSF receptor α subunit by electroporation. Cells selected by their resistance to G418 were sorted using phycoerythrin-labelled hGM-CSF to enrich for receptor expression. Analysis of hGM-CSF binding to cells that possessed the chimeric receptor revealed two separate classes of binding sites. The highest-affinity component had a kD of 3-5 x 10^{-9} M, which is similar to the low-affinity binding noted on normal cells. Cell surface expression of this component ranged from 50 to 300 sites/cell. Similar affinities were observed on cells transfected with the native GM-CSF receptor α subunit; however, the cell surface receptor expression generally was higher. Dose response curves of the FDCP1 and DA3 clones that contained the chimeric receptor showed that maximal cell proliferation (as measured by ^3H-thymidine incorporation) occurred at a GM-CSF concentration of 2 x 10^{-8} M. These results suggest that neither high-affinity binding nor the GM-CSF receptor cytoplasmic region is necessary for hGM-CSF-dependent signal transduction.

M 222 STUDIES OF PROGNOSTIC INDICATORS AND DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), Suzanne Kamel-Reid and Georgia Li, Oncology Research Program, Toronto General Hospital, and The University of Toronto Hospitals' Cancer Cytogenetics and Molecular Oncology Program, 100 College Street, Toronto, Ontario, Canada M5G 1L5
In CLL, survival varies anywhere from 10 or more years to less than 2 years. We cannot accurately predict which patients with the more common low- or intermediate-grade stages of CLL will have a disease that evolves to a more aggressive form. Our research goals are two-fold. First, we wish to define prognostic factors that may identify patients who are likely to progress to a higher grade disease. Better prognosticators will allow a better assessment of treatment modalities. Secondly, we wish to understand the pathogenesis of CLL by studying the mechanism(s) of disease progression. Because leukemic cells do not grow well outside of their natural microenvironment, studies are being done both in vitro and in vivo. We have previously used a murine model system to grow cells from patients with acute lymphoblastic leukemia and demonstrated that growth in vivo can predict clinical outcome (Science 246:1597, 1989; Blood, 1991). In our present studies leukemic cells from patients with CLL who are in different risk categories are being examined in vivo to determine if the rate and/or pattern of growth of these cells is correlated with clinical outcome. We are also examining the karyotype, phenotype and genotype of all leukemic clones grown both in vivo and in vitro. Data derived from studies of seventeen patients at different stages of CLL are currently being studied using these approaches. Multi-variate statistical analysis of the data generated, in concert with the in vivo growth patterns will allow an assessment of possible prognosticators associated with specific disease stages. Our results will be presented and discussed.

M 223 THE HUMAN PRE-B ALL TRANSLOCATION GENE, E2A-PBX1, INDUCES MYELOID LEUKEMIAS IN MICE. Mark Kamps* and David Baltimore#. *Department of Pathology, University of California, San Diego, La Jolla, CA 92093. # Rockefellor University, New York, NY, 10021. Twenty-five percent of pediatric pre-B cell leukemias contain the t(1;19) translocation, which joins the proven transactivation domain encoded by E2A with a homeobox, DNA-binding domain from the PBX1 gene. At least two E2A-Pbx1 fusion proteins arise by alternate splicing of t(1:19) transcripts: expression of either causes malignant transformation of NIH3T3 cells. In an attempt to understand the specific role of the chimeric E2A-Pbx1 protein upon hematopoietic differentiation in mice, we have performed marrow transplantation experiments where donor marrow, infected by viruses encoding E2A-Pbx1, is used to reconstitute the marrow compartment of a lethally irradiated recipient. In these experiments, all mice have contracted myeloid leukemias. Leukemic cells exhibited different stages of myeloid differentiation; some contained only myeloblasts, others myeloblasts with neutrophilic differentiation, and one, a strictly promyelocytic phenotype. One to two retroviral integration sites were present in each population of transformed myeloid cells, indicating that E2A-Pbx1-induced leukemia's were clonal. Six populations of tumor cells were grown in culture and characterized further. Immunoblot analysis with antibodies against Pbx1 confirmed that the correct size of E2A-Pbx1 was present in all samples, including those from maturing granulocytes. The behavior of these cells argue that E2A-Pbx1 itself does not induce factor-independence. Upon explant, all six leukemia-derived cell lines proliferated immediately in the presence of GM-CSF, whereas only one grew in 15% fetal bovine serum alone. Because five of the six cell types exhibit extensive death in the absence of lymphokine, E2A-Pbx1 alone does not substitute for growth factors. The behavior of E2A-Pbx1-induced leukemias demonstrates that E2A-Pbx1 does not completely block differentiation to granulocytes in response to normal cellular factors. Solid myeloid masses 0.85, 1.3, and 2.7 gms in size and containing myeloblasts as well as maturing and mature neutrophils were found in three animals. In addition, one animal had accumulated 5 ml of ascites fluid containing neutrophils and neutrophilic myelocytes. However, none of the tumor cells differentiated to either mature monocytes or neutrophiles when grown in the presence of GM-CSF, indicating that E2A-Pbx1 is consistantly associated with blocked differentaition in response to GM-CSF. These results suggest that oncogenes such as E2A-Pbx1 in human leukemias have the role of blocking differentiation in response to specific cellular factors that exist in marrow. Data illustrating the effects of E2A-Pbx1 upon differentiation of committed progenitors in tissue culture will also be presented.

M 224 EXPRESSION OF C-KIT IN T CELL PROGENITORS, Russel E. Kaufman, Carlos de Castro, Stephen M. Denning, G. R. Vandenbark, and Barton F. Haynes, Duke University Medical Center, Durham, N.C. 27710
The product of c-kit is a tyrosine kinase transmembrane receptor that is important for hematopoiesis in mice. Mutations in c-kit and in the gene that encodes the ligand of Kit, result in defects in stem cell migration and differentiation and the development of hereditary anemias. We have prepared a monoclonal antibody against the receptor Kit and have examined the distribution of this receptor on hematopoietic progenitor cells. Normal bone marrow and purified subpopulations were examined by FACS analysis and immunofluorescent staining using antibody 9B9. We demonstrated that 2-6% of normal bone marrow cells express Kit. The majority of these cells also expressed CD34. Study of bone marrow recovering from chemotherapy demonstrated a large and variable increase in the proportion of Kit+ cells. In addition we demonstrated a large increase in the CD7+ cells. We sorted normal thymocytes that were CD7+ and (CD4,CD8,CD3)-. Approximately 40% of these cells were Kit+. These triple negative CD7 positive cells have been demonstrated to be progenitors for T cells and have multipotent lineage capacity. Based on these studies, we conclude that Kit may be involved in the expansion or regulation of T cell progenitors.

M 225 FC GAMMA RECEPTOR EXPRESSION DURING MYELOPOIESIS J.M. Kerst,* A.H. Evans,* I.C.M. Slaper-Cortenbach,* C.E. van der Schoot,* A.E.G.Kr von dem Borne,[1,*] and M.H.J. van Oers.*[1] Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam and [1] Department of Hematology, Academic Medical Centre, Amsterdam, The Netherlands.
In this study we have examined phenotypic and functional characteristics that accompany the differentiation of committed progenitor cells along the myelomonocytic lineage. As a model of myelopoiesis, highly enriched progenitor cells were obtained by means of a fluorescence activated cell sorting procedure, in which the resulting cell population showed a 97% positive expression of the CD34 antigen. These cells were then stimulated with CSF in a suspension culture system and harvested at different time intervals, enabling growth dynamics and immunophenotypical markers of differentiation to be analysed. The proliferation measured in suspension by means the 3H thymidine incorporation correlated good to the number of CFU-GM as measured in semisolid medium (R=0.82). Flowcytometric immunophenotyping of the cells cultured in the presence of GM-CSF + G-CSF resulted in strong expression of the mature granulocytic antigens CD15 and CD67 (77.1±4.3 and 66.2±8.7). Differentiation to mature granulocytes could be confirmed morphologically and cytochemically. To examine Fc Receptor expression in this culture system we used immunorosettes in addition to flowcytometric immunophenotyping. The level of Fc Receptor expression (I,II and III) in the original CD34+ cell population were insignificant. As early as day 3 and 4 FcRI could be detected (30%) and reached a plateau of 60% by day 9, when stimulated with GM-CSF + G-CSF. FcRII and III were not detectable until day 5 and day 7 of culture, and ultimately rose to reach levels of 30% and 40% respectively. Fc Receptor expression was also functionally demonstrated by use of immunorosette binding and T-cell mitogenesis.
In conclusion, we have demonstrated that CD34+ cells do not express Fc gamma Receptors. FcRI is expressed early during myelopoiesis, preceeding the expression of FcRII and FcRIII.

M 226 TRIPLE NEGATIVE (CD3-4-8-) HUMAN THYMOCYTES EXPRESS CD4 AND CD8 FOLLOWING IN-VIVO AND IN-VITRO DIFFERENTIATION,
Daniel Kraft, Anis Sen Majumdar, Miriam Lieberman, Irving L. Weissman, Edmund K. Waller, Laboratory of Experimental Oncology; Department of Medicine, Stanford University Medical Center; Stanford, CA 94305-5468

Human T-Cell development and differentiation have been studied both in-vivo, utilizing heterologous human thymus xenografts in SCID-hu mice, and in-vitro, utilizing heterologous human thymic stromal cell cultures. T-Cell precursors were isolated from fetal thymus tissue by magnetic bead depletion of CD4+CD8+ cells, followed by FACS sorting for triple negative (TN) CD4-CD8-CD3- cells. For in-vivo studies, isolated TN cells were stained with the fluorescent membrane dye, PKH2. These cells were then injected into the thymic grafts of SCID-hu mice. 24-62 hours later, the grafts were removed, stained with monoclonal antibodies to T-cell surface antigens, and analyzed by FACS. Gating for PKH2 labelled cells revealed differentiation of TN cells to both CD4+ and CD8+ single and double positive populations. Parallel experiments were performed using allogeneic fetal thymic stroma cell cultures to assess in-vitro differentiation of T-cell precursors. Sorted TN cells failed to differentiate when placed in medium alone; after 48 hours all thymocytes had died. In contrast, sorted TN cells cultured for 48 hours on fetal thymic stroma differentiated mainly into CD4+CD8+ cells, with smaller numbers present in the CD4+CD8- and CD4-CD8+ populations. The kinetics of T-cell differentiation and the pattern of T cell antigen expression were similar in both the in-vivo and in-vitro experiments, suggesting these model systems may accurately reflect the events of normal T cell differentiation.

M 227 PHENOTYPIC AND FUNCTIONAL ABNORMALITIES OF BONE MARROW FROM NON-OBESE DIABETIC MICE.
P.B. Langmuir, M.M. Bridgett, A.L.M. Bothwell, and I.N. Crispe, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510.
Bone marrow cells from non-obese diabetic (NOD) mice were examined for the presence of phenotypic and functional abnormalities. Flow cytometry was used to determine the expression of various cell surface markers on NOD bone marrow cells: no differences were seen between NOD and BALB/c mice in the expression of Thy-1, Pgp-1/CD44, GF1, B220, or HSA, but NOD mice displayed markedly decreased expression of both Ly-6C and AA4.1. Multilineage progenitor cell activity was found to be identical in NOD and BALB/c bone marrow; however, bone marrow cultures with different growth factors demonstrated a defect in IL-3 responsive progenitor cells in NOD mice; this defect was not seen in BALB/c, ST/b, or C57BL/6 bone marrow cultures. The poor response of NOD bone marrow cultures to IL-3 was seen when either supernatant from WEHI-3 cells (a source of IL-3) or recombinant IL-3 was used as the growth factor, but there was no decrease in the total number of NOD colonies responding to either recombinant GM-CSF or supernatant from L929 cells (a source of M-CSF). When colony morphology was examined, a population of dense-centered colonies was found to be strikingly reduced in cultures of NOD bone marrow with either IL-3 or GM-CSF. These dense-centered colonies were shown to reduce the dye nitroblue tetrazolium, thus suggesting that a population of myeloid precursors in NOD mice is defective in its response to IL-3 or GM-CSF.

M 228 GROWTH FACTOR INDUCED PROLIFERATION OF HEMATOPOIETIC PROGENITOR (CD34+) CELLS FROM ADULT BONE MARROW AND CORD BLOOD: EVALUATION OF THE FIRST THREE CONSECUTIVE CELL CYCLES.
Filip Lardon, Dirk R. Van Bockstaele, Hans-W. Snoeck and Marc E. Peetermans, Laboratory for Experimental Hematology, University of Antwerp (U.Z.A.), Belgium.

A limitation shared by most methods to evaluate cell kinetics to date is that growth of cells beyond the first cell cycle cannot be further discriminated.
The BrdU-Hoechst quenching technique however can resolve three succesive cell cycles. In addition, cell cycle arrest in any of the phases can be revealed.
We used this technique to study the kinetic response of highly purified hematopoietic progenitor cells (CD34+ sorted fraction) to growth factors. To compare the in vitro proliferative potential of adult to foetal progenitors, cells were harvested from normal bone marrow and cord blood respectively.
In serum supplemented cultures, a marked proliferation of the progenitors was seen even without addition of growth factors, suggesting the presence of stimulatory factors in the serum. We therefore used serum deprived culture conditions to evaluate growth factor induced proliferation.
The combination of GM-CSF plus G-CSF, acting synergistically in day 14 cultures, was found to have a less than additive effect in the first three cell cycles. Some target cells may therefore become responsive at a later stage.
Furthermore, we examined the proliferative effects of IL-3 and IL-6. When compared to IL-3 alone, the combination of IL-3 plus IL-6 was observed to induce a more rapid expansion during the first three cell cycles.

M 229 IMPORTANCE OF p56^{lck} ASSOCIATION WITH CD4 AND CD8 DURING THYMOCYTE DEVELOPMENT,
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The product of the *lck* proto-oncogene (p56^{lck}) is a membrane-associated protein tyrosine kinase preferentially expressed in T lymphocytes. It is normally found in association with the CD4 and CD8 coreceptors and thus is circumstantially implicated in signal transduction from the lymphocyte surface. To study the nature of this signal, we sought to determine the effects of increased p56^{lck} kinase activity during thymocyte development. By over-expressing the wild-type (LGY) or an activated form of the kinase (LGF) in thymocytes, we were able to perturb thymic development in a predictable fashion dependent on *lck* kinase activity. Animals expressing high levels of the transgenes developed thymic tumors while animals expressing lower levels showed a reduced number of surface CD3$^+$ thymocytes, exhibited retarded thymic development, and produced fewer mature T cells. The severity of these defects were correlated with transgene expression levels. To determine which, if any, of these abnormalities were the result of a CD4- or CD8-mediated signal through p56^{lck}, transgenic animals were generated over-expressing an activated form of the *lck* gene containing mutations in two amino-terminal cysteine residues known to be essential for interaction of the kinase with CD4 and CD8 (LGCAF). Analysis of these animals indicates that all of the phenotypic effects induced by excess p56^{lck} activity in thymocytes are independent of the association of p56^{lck} with CD4 and CD8.

M 230 THE ROLE OF THE HOMEOBOX GENE, HOX 2.3, IN MONOCYTE DIFFERENTIATION AND T-CELL ACTIVATION,

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Homeobox genes encode proteins containing a 61-amino acid "homeodomain" that binds DNA in a helix-turn-helix structure. These genes are involved in the coordinated regulation of expression of structural genes involved in development. Although originally described as playing a crucial role in embryogenesis, it is becoming increasingly evident that homeobox genes are also involved in differentiation processes in the mature organism. We have studied a specific homeobox gene, Hox 2.3, because it was reported to be expressed in a lineage-restricted fashion in cells of the monocyte/macrophage phenotype (Kongsuwan, *EMBO J* 7:2131-2138, 1988). Flanking primers to the 5' and 3' coding regions of human Hox 2.3 were designed from the published sequence, and reverse PCR was performed using RNA from a variety of human hematopoietic cell lines. PCR products were subcloned into the TA vector and sequenced to confirm identity with Hox 2.3. We have confirmed and extended the previous report, showing absence of constitutive expression in myeloid cell lines, HL-60 and KG-1, and constitutive expression in the monocytic cell line, THP-1. Preliminary anti-sense oligonucleotide experiments suggest that Hox 2.3 expression is necessary for induction of monocytic differentiation in HL-60 cells by chemical inducers. It was recently reported that Hox 2.3 is also expressed in human B- and T-cell lines (Deguchi et al., *Blood* 78:445-450, 1991). In our studies, Hox 2.3 RNA was undetectable in resting T-cell lines, but expression was induced with PHA and TPA, mimicking T-cell activation. In addition, constitutive expression of Hox 2.3 was observed in some human cell lines infected with the retrovirus, human T-cell leukemia virus type I. Taken together, these results suggest a role for homeobox genes in the control of lineage commitment decisions in the mature organism, as well as during morphogenesis.

M 231 STROMAL-CELL DEPENDENT GROWTH OF HUMAN FETAL B CELL PRECURSORS.

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We have recently shown that fetal B cell precursors (CD10^{+}sIgM) proliferate in response to recombinant human IL-7 but this does not result in long-term cell expansion. Therefore, we have examined whether coculture of B cell precursors in the presence of stromal cells would enhance this observed proliferative response. Human fibroblastic stromal cells derived from adult bone marrow were found to sustain the growth of fetal CD10^{+} sIgM^{-} B cell precursors. In particular, in the presence of exogenous IL-7, cultures could be kept up to three to four weeks, and resulted in a 4 fold expansion in cell numbers, when compared to input values.

When compared with other adherent cell lines, bone marrow stroma was the only one that supported BCP proliferation.

Cells generated in the cultures remained at the precursor stage, as only few sIgM^{+} cells were found to appear. We are now trying to find culture conditions that will allow the maturation to sIgM^{+} sIgD^{+} human B cells.

M 232 HUMAN MARROW $CD7^{+}CD2^{-}CD34^{-}$ CELLS ARE COMMITTED TO T CELL LINEAGE: POSSIBLE FILIATION FROM EARLIER $CD34^{+}CD7^{+}$ PRECURSOR.

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Earlier detectable human thymic cells are $CD7^{+}2^{-}3^{-}4^{-}8^{-}$ (pro T) and can generate $CD2^{+}CD3^{+}$ cells following appropriate in-vitro conditioning. These cells are mostly localized within the outer cortex region of the thymus and do not express CD34 antigen. These pro T cells ($CD7^{+}34^{-}2^{-}$) are also detected in human bone marrow with similar in-vitro features as thymic precursors. In addition, our data suggest that human marrow cells contain $CD7^{+}34^{+}$ cells which, by contrast to pro T cells, display both lymphoid and myeloid potentials. In vitro, we failed to generate lymphoid cells from $CD34^{+}7^{-}$ precursors. However, early study in SCID mice pointed to higher reconstitution capacity of $CD34^{+}7^{-}$ precursors as compared to $CD34^{+}7^{+}$ cells. Together, these data suggest that $CD7^{+}$ cells development from earlier $CD34^{+}$ multipotential precursors and their commitment to T cell lineage occur in human bone marrow.

M 233 DELETION AND REPLACEMENT OF THE IGH INTRON ENHANCER BY HOMOLOGOUS RECOMBINATION VIA THE HIT & RUN PROCEDURE,

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Enhancers have been defined as cis-regulatory elements which control the transcription of nearby genes. In the case of the intron enhancer ($E\mu$) of the Ig heavy chain (H) locus many sequence motifs have been defined which are bound by regulatory proteins, some of which are identified as transcription factors. However, it appears that the intron enhancer could fulfil additional functions different from the transcriptional control. For example, experiments by P. Ferrier et al. (EMBO J. **9**, 117 [1990]) suggested that $E\mu$ act as a cis-regulatory element in the control of variable gene segment (V[D]J) recombination. To elucidate the functions of the IgH intron enhancer during B cell development *in vivo* we want to establish mice which either lack the intron enhancer or carry heterologous enhancer within the IgH locus. Using the "Hit & Run" procedure developed by Hasty et al. (Nature **350**, 243 [1991]) my laboratory has established targeted ES cell lines and is currently generating chimeric animals.

M 234 GM-CSF-RESPONSIVE SEQUENCES UPSTREAM OF THE PRIMARY RESPONSE GENE, EGR-1/TIS8, IN A HUMAN FACTOR-DEPENDENT MYELOID LEUKEMIA CELL LINE,

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) promotes the proliferation and maturation of myeloid progenitor cells and enhances the function of neutrophils, monocytes and eosinophils. The specific biochemical pathways mediating GM-CSF action are unknown. We have demonstrated that GM-CSF induces rapid and transient induction of the primary response gene, EGR-1/TIS8, in a murine myeloid leukemia cell line (32D) and human neutrophils (Varnum et al., *Mol Cell Biol* 9:3580-3583, 1989). In order to identify the sequences mediating GM-CSF-induced gene expression, we isolated and mapped the human EGR-1/TIS8 gene and sequenced approximately 700 nucleotides (nt) of the 5' upstream region. This promoter region contains several putative regulatory elements, including SPI binding sites, an AP-1 site, serum response elements, an EGR-1 binding site, and a cAMP-responsive element. A 600-nt fragment of this promoter and several deletion mutants were subcloned into a vector containing the chloramphenicol acetyltransferase (CAT) gene. These constructs were transiently transfected into a human factor-dependent myeloid leukemia cell line, TF-1. GM-CSF stimulated CAT expression from the full-length -600 construct six-fold (range of four- to ten-fold) over that found in unstimulated cells. Deletion to nt -480 reduced this induction to four-fold. Further deletion to nt -235 abolished inducible promoter activity. Additional constructs are being employed to further characterize GM-CSF-responsive sequences. By defining early nuclear events following GM-CSF stimulation of factor-dependent cells, we can compare signal transduction pathways of other growth factors such as interleukin 3 (IL-3) and granulocyte colony-stimulating factor (G-CSF), which have known overlapping biological effects with those of GM-CSF.

M 235 EXPRESSION OF C/EBP-RELATED TRANSCRIPTION FACTORS CORRELATES WITH THE LINEAGE SWITCH OF TRANSFORMED B CELLS,

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Transformed B cells can undergo a lineage conversion to a monocyte/macrophage phenotype. Cells that undergo this conversion become adherent, gain an extensive vacuolated cytoplasm, express high levels of Mac-1 and α-naphthyl acetate esterase, and acquire the capacity to phagocytose, process and present antigen. This process is accompanied by the down regulation of *c-myc* and *c-myb*, two proto-oncogenes that act as transcription factors. Here we report the up regulation of the CRP2 and CRP3 transcription factors in "lineage switch" cells. CRP2 (also reported as NF-IL6, LAP, IL-6DBP and AGP/EBP) and CRP3 belong to a C/EBP-like protein family, and exhibit DNA-binding and leucine zipper dimerization specificities that are nearly identical to those of C/EBP. Previous studies suggested that CRP2 is a regulatory component of the acute phase response in hepatocytes. In accordance with this role, treatment of cells with interleukin 1 or 6 was seen to elicit an increase in CRP2 binding activity. Although Northern blot analysis revealed that CRP2 and CRP3 RNAs are expressed in a wide variety of tissues, we show that its expression within the hematopoietic lineages is restricted to macrophages. CRP2 protein experssion has also been detected in "lineage switch" macrophages, as well as changes in the gel shift profiles obtained from incubation of nuclear extracts with C/EBP binding motifs. The strict correlation between CRP2 mRNA expression and the macrophage phenotype, observed in several independent cell lines, suggests that CRP2 may also have a function in determining macrophage differentiation. Experiments are underway that examine the consequences of ectopic expression of CRP2 in B lymphoid cells.

M 236 IN VITRO DIFFERENTIATION OF CD3⁻4⁻8⁻ THYMOCYTES INTO PHENOTYPICALLY MATURE CELLS IN THE PRESENCE OF THYMIC STROMAL CULTURES.

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Interaction of thymocytes with thymic stromal cells influences the differentiation and T cell receptor repertoire selection of maturing thymocytes. We have investigated the ability of heterogeneous thymic stromal cell (HTSC) culture system to promote in vitro differentiation of $CD3^-4^-8^-$ thymocytes as well as of a subset of these cells identified by the high expression of 1C11 antigen. Culture of purified murine $CD3^-4^-8^-$ (TN) thymocytes on HTSC for 1 day resulted in appreciable increase in the percentage of $CD4^+8^+$ thymocytes while keeping the sorted cells in medium alone failed to generate $CD4^+8^+$ cells. When the culture period was extended to 2 days, TN cells were found to further differentiate into $CD4^+8^-$ and $CD4^-8^+$ cells which also expressed high levels of the CD3 antigen. We next examined the differentiation potential of a subset of TN thymocytes which express high levels of 1C11 antigen. Similar to total TN thymocytes, $1C11^{hi}$ TN cells were also able to give rise to $CD4^-8^+$ and $CD4^+8^+$ cells after one day of culture on HTSC and most of these cells were $CD3^-$. However, on extending the culture period to 2 days resulted in a significant percentage of $CD3^+$ cells which were $CD4^+8^+$, $CD4^+8^-$ and $CD4^-8^+$ cells. These results suggest that in our in vitro HTSC culture system, immature TN thymocytes can differentiate into all of the mature phenotypes of cells normally found in the adult mouse thymus.

M 237 LYAR, A NEW ZINC FINGER PROTEIN INVOLVED IN LEUKEMOGENESIS AND EARLY DEVELOPMENT

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Lyar was cloned from a mouse T cell leukemia cell line. Computer-aided homology searches revealed that it had two zinc fingers. Lyar was expressed at high level in all the T and B leukemia cell lines tested. It is also present in early fetal tissues but not in adult tissues with the exception of expression in the immature germ cells in the testis. Finally, blocking lyar function in leukemic cell line can inhibit its growth. Therefore, lyar is involved in some aspects of leukemic cell growth. DNA blot analyses indicated that lyar-related sequences are present in human, rat and chicken. So the lyar function may be conserved during evolution.

We are currently investigating the function of Lyar by retrovirus-mediated gene transfer and by transgenic animal models. Experiments are also in progress to identify the specific DNA sequences which bind to the lyar zinc fingers. This will help to identify the target genes of the lyar action, and therefore its functions..

M 238 S6 KINASES IN MODELS OF T CELL RECEPTOR ACTIVATION AND PROLIFERATION, Terry A. Vik, Craig M. Crews, Victor Calvo, and Barbara E. Bierer, Department of Pediatrics, H.B Wells Center for Pediatric Research, IU School of Medicine, Indianapolis, IN 46202, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, and Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 02115

We have measured S6 kinase activities in cells which are models of T cell activation and proliferation. The ribosomal protein S6 kinases are mitogenically activated indirectly through stimulation of membrane associated tyrosine kinases. Members from each of the two major S6 kinase families, $pp70^{S6K}$ and $pp90^{rsk}$, have been cloned. The Jurkat cell line was used to study S6 kinase activation in response to T cell activation through the T cell receptor-CD3 complex. Antibody which crosslinks the T cell receptor-CD3 complex (TCR) does not increase total S6 kinase activity. However, when $pp90^{rsk}$ is measured in an immunocomplex assay, its activity rapidly increases, peaking 5 minutes post-stimulation.

The basal S6 kinase activity in Jurkat cells appears to be due to $pp70^{S6K}$. Extracts from unstimulated cells, resolved by ion exchange chromatography show a peak of basal S6 kinase activity. Following stimulation through the TCR, cell extracts, resolved by ion exchange chromatography show two peaks of S6 kinase activity. The earlier eluting peak corresponds to $pp90^{rsk}$ activity which can be demonstrated by immunocomplex assay using anti-$pp90^{rsk}$ sera. The second peak corresponds to basal S6 kinase activity and does not increase with TCR activation.

Activation of resting T cells by stimulation through the TCR sets in motion a series of events leading to expression of the IL-2 receptor, production of IL-2, and clonal proliferation of activated T cells. The IL-2 dependent cell line CTLL was used to study the role of S6 kinases in IL-2 mediated signal transduction. Cells were starved of IL-2 for 3 hours prior to re-stimulation with IL-2. Cell extracts were prepared at time points following re-stimulation. Total S6 kinase activity increased by 5 minutes post stimulation and reached a steady state level by 30 minutes. No change in activity due to $pp90^{rsk}$ as measured by immunocomplex assay was noted. These data suggest roles for $pp90^{rsk}$ in signalling early events in T cell activation through the T cell receptor-CD3 complex, and for $pp70^{S6K}$ in continued proliferation of T cells in response to IL-2.

M 239 HUMAN T-CELLS FROM SCID-HU MICE DEMONSTRATE ANTIGEN-SPECIFIC DELETIONS, PROLIFERATION AND ANERGY FOLLOWING EXPOSURE TO STAPHYLOCOCCAL ENTEROTOXINS, Edmund K.Waller , Anis Sen Majumdar, Gun A. Hansteen, Michael R. Schick, Onsi W. Kamel , and Irving L. Weissman, Laboratory of Experimental Oncology; Department of Medicine, Stanford University Medical Center; Stanford, CA 94305-5468

SCID-hu mice provide an in vivo model for studying the events of normal human T-cell development and differentiation. We treated SCID-hu mice with staphylococcal enterotoxins (SE) and determined their effects on the development of clonotypic human T-cell populations, defined by their expression of V beta molecules in their T-cell receptors. Following a single dose of SEB we observed a specific effect on the pattern of V beta 12.1 expression on thymocytes while other clonotypic T-cell populations (V beta 5.2, V beta 5.3, V beta 8.1) remained unchanged. There was a transient decrease, over 6 to 48 hours, in the proportion of CD4+8- thymocytes that were V beta 12.1+, and a corresponding increase in the fraction of CD4-8+ thymocytes that expressed V beta 12.1. There was no effect on the total percentage of thymocytes that expressed any specific V beta molecule, nor in the fractions of the CD4+8- or CD4-8+ subpopulations that expressed V beta 5 or V beta 8.1. There was a minimal effect on the total numbers or relative proportions of CD4+8- or CD4-8+ cells following SEB treatment. Treatment of SCID-hu mice with multiple injections of SEB resulted in larger effects on the distribution of V beta 12.1+ cells than a single injection, without producing changes in the numbers of CD4+8-, CD4-8+, V beta 5.2+ or V beta 8.1+ cells. Treatment of SCID-hu mice with a related enterotoxin, SEE, resulted in a similar reduction of V beta 8.1+ CD4+8- thymocytes and an increase in V beta 8.1+ CD4-8+ thymocytes without significant effect on V beta 5.2/5.3+ or V beta 12.1+ sub-populations. Thymocytes prepared from SEB-treated SCID-hu mice demonstrated specific anergy to SEB but not to SEA, SEC3 or SEE in an in vitro proliferation assay, while thymocytes prepared from SCID-hu mice exposed to SEE in vivo were anergic to SEE but not to other superantigens. Continuous exposure of thymocytes to SEB in vitro resulted in the specific deletion of V beta 12.1+ CD4+8- cells, while in vitro treatment with SEE produced a deletion of V beta 8.1+ cells of the same phenotype. Peripheral blood leukocytes prepared from SEB-treated SCID-hu mice contained 5-10 fold increased numbers of V beta 12.1 CD4+8- T-cell 3 and 4 days following a single injection of SEB, compared to sham injected SCID-hu control mice. Using staphylococcal enterotoxins as superantigens in the SCID-hu system, we have been able to demonstrate antigen specific clonal deletion, anergy and proliferation of human T-cells.

M 240 SUPRESSION OF ABNORMAL T CELL DEVELOPMENT IN GLD MICE BY TRANSGENIC T CELL RECEPTOR BETA CHAIN, Katusyuki Yui, Avinash Bhandoola, Shinji Komori, Makoto Katsumata, and Mark I. Greene; Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

Mice homozygous for the gld (generalized lymphproliferative disorders) mutation develop severe lymphadenopathy due to an age related accumulation of polyclonal T cells in the peripheral lymphoid organs. The majority of these T cells have a unique phenotype reminiscent of immature T cells: they are $CD4^-CD8^-TCR\alpha\beta^+$, overexpress TCR $\alpha\beta$ chain RNA, proto-oncogenes c-myb and fyn, and proliferate poorly to TCR mediated stimulation. To study the influence of a functionally rearranged TCR β chain on the T cell developmental abnormality of the gld mutation, we have backcrossed TCR Vβ8.1 transgenic mice (tg.) to C3H-gld/gld to obtain transgenic mice homozygous for the gld mutation (tg. gld mice). Surprisingly, lymphadenopathy was markedly suppressed and $CD4^-CD8^-$ T cells did not accumulate in tg. gld mice. However, the remaining T cells in tg. gld mice overexpressed c-myb at a mild level and proliferated poorly to TCR occupancy. These features indicate that the abnormal gene expression pattern and function persists in phenotypically normal T cells in gld mice, and that these characters can be dissociated from the accumulation of $CD4^-CD8^-$ T cells. To further investigate the mechanisms underlying the disappearance of $CD4^-CD8^-$ T cells in tg. gld mice, fetal ontogeny of T cells was compared in tg. and non-transgenic mice. In the tg. thymus, development of $TCR\alpha\beta^+$ cells was advanced as detected by early expression of CD4, CD8 and TCR. In contrast, the number of $TCR\gamma\delta^+$ cells was reduced. Correlation of these features with the reduction of $CD4^-CD8^-$ T cells in tg. gld mice is under investigation.

Myeloid Growth Factors II & III

M 300 THE 5' FLANKING REGION OF C10

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C10 is a recently described member of the MIP-1 cytokine family expressed in normal mouse bone marrow cells and in several mouse myeloid cell lines. The 5' flanking region of C10 has been cloned and sequenced and the transcription initiation site determined by primer extension. The 1400 bp 5' flanking region contains a TATA box at position -31, and does not contain sequences homologous to the myb binding site or to the 5' flanking region of myeloid specific proteins such as myeloperoxidase. C10 appears to be transcriptionally induced in mouse bone marrow cells stimulated to differentiate with GM-CSF, as C10 heteronuclear RNA is induced. The C10 5' flanking region demonstrates promoter activity when introduced in a luciferase construct into P388D1 cells, a macrophage cell line that expresses C10. Transcriptional regulation of C10 may be important in the control of myeloid differentiation.

M 301 THE ACTIVATION OF INACTIVE MEMBRANE PROTEIN KINASE C IS ASSOCIATED WITH DMSO-INDUCED ERYTHROLEUKEMIA CELL DIFFERENTIATION, Balu R. Chakravarthy, Roger Tremblay, Philip Macdonald, James F. Whitfield, and Jon P.Durkin, Cell Signals Group, Institute for Biological Sciences, National Research Council of Canada, Ottawa, K1A 0R6, Canada.
It has been suggested that the redistribution of cytosolic PKC to membranes, and its subsequent proteolytic activation to PKM is necessary for the DMSO/HMBA-induced differentiation of murine erythroleukemia (MEL) cells. However, in a specific MEL cell subline (MEL1), DMSO did not induce any detectable change in PKC distribution, even though the cells differentiated normally in response to the agent. Nevertheless, the differentiation of MEL1 cells was partially blocked by the PKC inhibitor H-7 when the inhibitor was added only within the first 2 hours after DMSO induction, indicating that an early PKC-dependent event was necessary for the process. A 2- to 3-fold increase in PKC activity was detected in membranes isolated from DMSO-treated MEL1 cells when enzymatic activity was measured by a recently developed method which quantitates the amount of 'active' PKC in intact membranes. This transient increase, which occurred within 15-60 min after DMSO addition, resulted from the activation of an 'inactive' pool of the enzyme already associated with membranes, and not from the translocation of cytosolic PKC. Conventional PKC assays are unable to detect such changes in the inactive/active membrane PKC pool. DMSO also caused a rapid increase in nuclear PKC activity in MEL1 cells. Similar increases in membrane PKC activity were observed in both HEL human erythroleukemia cells and HL-60 human myeloleukemia cells induced to differentiate by DMSO, but not in S49T-lymphoma and WEHI-231 B-lymphoma cells which do not undergo apparent differentiation. The results suggest that a rapid and transient increase in membrane PKC activity may be an important early step in DMSO induced-differentiation of erythroleukemia cells. These findings will be discussed in light of related studies in our laboratory which indicate that the activation of a preexisting pool of inactive membrane PKC, rather than a TPA-like translocation of cytosolic enzyme, may be the preferred mechanism by which many growth factors and hormones stimulate membrane PKC activity.

M 302 LONG-TERM RECONSTITUTION WITH BONE MARROW CELLS INFECTED WITH RETROVIRUS EXPRESSING INTERLEUKIN 4 . Cynthia A. Chambers, Joonsoo Kang, and Nobumichi Hozumi, Division of Neurobiology & Immunology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Dept. of Immunology, University of Toronto, Toronto, Ont., Can., M5G 1X5.
Interleukin 4 (IL-4) is a pleiotropic cytokine secreted by subsets of activated T cells. IL-4 plays an important role in regulating the nature of the immune response generated against antigen, Ig isotype expression and in thymocyte differentiation. In order to study the role of IL-4 on T cell regulation of immune responses in vivo, a high titer recombinant retroviral vector (>10^7cfu/mL) containing mouse IL-4 cDNA under the control of the internal β-actin promoter/enhancer was used to infect bone marrow cells. Lethally irradiated mice were reconstituted and analyzed 3-8 months post-transfer. Eleven out of 12 mice tested expressed retroviral IL-4 mRNA, and IL-4 protein was detectable at physiologically relevant levels in supernatants from unstimulated splenocytes and thymocytes. Several phenotypic changes were observed as a result of ectopic IL-4 expression, including changes in serum Ig isotype concentrations and thymocyte differentiation. Some of these changes were detected in mice expressing low levels of IL-4. Using this model system, mechanisms of non- or low-responsiveness to antigens in inbred mouse strains of mice can be examined, and the feasiblity of cytokine gene therapy can be evaluated. (Supported by MRC and NCI, Canada)

M 303 MAST CELL GROWTH FACTOR ENHANCES RETROVIRAL VECTOR-MEDIATED GENE TRANSFER INTO HUMAN HEMATOPOIETIC PROGENITORS, Gay M. Crooks, Jan A. Nolta, Robert W. Overell, Douglas E. Williams and Donald B. Kohn, Division of Research Immunology and Bone Marrow Transplantation, Childrens Hospital of Los Angeles, Los Angeles, CA 90027
Retroviral vector mediated gene transfer into human hematopoietic stem cells (HSC) has potentially important implications for gene therapy, but has proven technically difficult. Successful transduction requires active cycling of target cells. HSC exist predominantly in G_0 and this low rate of division may be a significant factor limiting the efficiency of gene transfer. Hematopoietic growth factors (HGF) are known to stimulate cycling of, and increase gene transfer into, early hematopoietic progenitors. We have examined the effects on gene transfer of Mast Cell Growth Factor (MGF) when used alone or in various combinations with IL-1,3 and 6 to prestimulate CD 34(+) bone marrow (BM) cells. In all experiments, BM was cocultivated with fibroblasts packaging the LN vector which carries the gene for neomycin resistance. Gene transfer was assayed by %G418 resistance in colony forming units (CFU). The combination of IL-1,3,6 and MGF produced 40-50% G418 resistance in CFUs and long term culture initiating cells (LTCIC) compared to 5-10% G418 resistance in media without growth factors ("basal") and 30-40% with IL3 plus IL6. Prolonging prestimulation and cocultivation for up to 7 days did not improve gene transfer further. To study the effects of these HGF on a more primitive subpopulation of CD34(+) cells, we used BM purged with 4-hydroperoxycyclophosphamide (4-HC), an agent toxic to more committed progenitors. 4-HC resulted in depletion of 99.5% of the progenitors capable of forming colonies after 14 days. The combination of IL-1,3,6 and MGF produced the highest efficiency of gene transfer into day 30 CFUs (27% vs 17% with IL3 plus 6). These results suggest that MGF, when used in combination with other HGF, increases gene transfer efficiency into human primitive hematopoietic progenitors.

M 304 REGULATION OF M-O7e CELL PROLIFERATION AND DIFFERENTIATION: ROLE OF NF-E1, Wei Dai, Mao Huang, Phillip Daschner, Connie Erickson-Miller, Ralph Parchment, Martin J. Murphy, Jr. Hipple Cancer Research Center, 4100 South Kettering Boulevard, Dayton, OH 45439

Erythroid differentiation is primarily mediated by erythropoietin (Epo) though interleukin-3 (IL-3) and erythroid-potentiating activity (EPA) also play an important role in erythroid proliferation and maturation. The interaction of Epo with its specific membrane receptor on target cells triggers a series of cellular events that lead to activation of pre-existing protein factors or new gene expression, which in turn stimulate committed erythrocyte precursor cells to survive, proliferate, and differentiate toward mature erythrocytes. The M-O7e human megakaryoblastic leukemic cell line has a strict requirement for IL-3 or GM-CSF for survival and proliferation. Since the cells express surface membrane determinants found in bipotent erythro-megakaryocytic cells (CD36, H25), we tested if Epo has an effect on M-O7e cells in terms of their proliferation or differentiation. Epo treatment significantly inhibits M-O7e growth either in the presence or absence of IL-3. Using mobility shift analysis we detected in highly proliferative M-O7e cells a low activity of NF-E1, a transcription factor found in committed erythrocytic and megakaryocytic cell lineages. The specificity of the binding was confirmed by competition with excess amount of the unlabelled specific DNA fragment. We further asked if Epo-induced decrease in M-O7e growth rate is correlated with a change of NF-E1 activity. After Epo treatment NF-E1 activity in M-O7e cells increased substantially and this activity appeared to be synergized by cotreatment of the cells with dimethyl sulfoxide (DMSO), a chemical agent found to cause murine erythroleukemic cells to differentiate by induction of Epo receptor. Compared with K562 human erythroleukemic cells, NF-E1 activity detected in M-O7e cells was lower after treatment with the inducing agents of Epo and DMSO. In addition to NF-E1 activity, we also detected in M-O7e, K562 and Daudi lymphoblastic leukemic cells a second specific DNA binding factor with a faster mobility. The role of this new DNA binding activity in relation to NF-E1 in regulating erythroid and/or megakaryocytic specific gene expression is under current investigation.

M 305 IDENTIFICATION OF THE REPERTOIRE OF NON-SRC-RELATED TYROSINE KINASES EXPRESSED IN RESTING CD34 CELLS. J.F. DiPersio, S. Luhowskyj, J. Casnelli University of Rochester Medical Center, Rochester, NY.

Highly degenerative oligonucleotide primers to domain VI (sense strand) and domain IX (antisense) (Hanks et al. Science 241:42, 1988) of the human protein tyrosine kinase family were designed. These degenerate primers were used to PCR cDNA obtained from resting (unstimulated) highly purified $CD34^+$ human marrow stem cells. In excess of 100 independent partial cDNA clones have been examined by either complete or partial (single base) sequencing. Of interest is that in spite of significant sequence similarity between both SRC-like and non-SRC tyrosine kinases and between serine/threonine kinases in this region, only non-SRC tyrosine kinases could be identified. The most frequently identified sequences were those of insulin growth factor-1 receptor >cfms >c-fps/c-fes > c-met >c-fer. At least 6 novel human CD34 tyrosine kinase partial cDNA clones have been characterized and sequenced in addition to the janice kinase (JAK1) recently shown to contain both ser/thr as well as tyrosine kinase domains. These unique clones possess varying degrees of both nucleic acid and AA homology to several other tyrosine kinases including c-met, CSK, and TRK the nerve growth factor receptor. We are presently attempting to both isolate full length clones encoding these novel TK's as well as to study their developmental regulation during hematopoietic development.

M 306 MUTATIONAL ANALYSIS OF STRUCTURE-FUNCTION RELATIONSHIP OF THE HUMAN GM-CSF RECEPTOR, Parul D. Doshi, John F. DiPersio, Hematology unit, University of Rochester, Rochester, NY 14642.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 22Kd glycoprotein which stimulates proliferation, differentiation and functional activation of granulocytes and macrophages. The human GM-CSF receptor (GM-R) consists of two subunits, the α subunit constitutes the low affinity receptor and the β subunit, when complexed with the α subunit constitutes the high affinity receptor. The GM-R(α) shares some highly conserved structural features with other members of the hematopoietin receptor superfamily such as IL-3, IL-4, IL-7 and EPO-R. We have proposed to systematically alter the conserved sequence motifs or structural domains crucial for receptor function and examine the effect on ligand binding and transmembrane signaling. We have altered the following three conserved sequence motifs by oligonucleotide-directed *in vitro* mutagenesis: (1) RW (nt # 860, trp to cys) (2) WSXWS (nt # 1075, trp to leu) (3) Cys in TM domain (nt # 1160, cys to ser). Specific regions of the ligand binding domain and the cytoplasmic domain were deleted by standard molecular manipulations to determine which of these regions are involved in ligand binding and signal transduction respectively. These constructs were subcloned into retroviral mammalian expression system to facilitate the expression of the mutant GM-R in factor- dependent and independent cell lines. We are now examining the consequences of some of the mutations by transfecting the mutant cDNAs into COS cells. The expression of mutant constructs in transfected cell lines was confirmed by PCR and routine northern blot analysis. The effects of these mutants on receptor function are studied by binding of ^{125}I-GM-CSF to transfected cells. The effect of some of these mutations on ligand-binding will be presented.

M 307 TRANS-REGULATION OF M-CSF RECEPTOR EXPRESSION DURING MYELOID CELL DEVELOPMENT. Brian C. Gliniak, and Larry R. Rohrschneider, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Development of specialized myeloid cells is mediated by the actions of a hierarchy of hematopoietic growth factors and the coordinated expression of their receptors. We have previously reported the isolation of a murine myeloid precursor cell line, designated FDC-P1/MAC, that simultaneously expresses receptors for multi-CSF, GM-CSF, and M-CSF. Growth of FDC-P1-MAC cells in either multi-CSF or GM-CSF results in the post-transcriptional suppression of M-CSF receptor (c-*fms* proto-oncogene) expression. We use the term trans-regulation to describe this control of receptor expression and have further characterized this regulatory process. The removal of FDC-P1/MAC cells from GM-CSF stimulation resulted in the re-expression of c-*fms* mRNA independent of new protein synthesis and M-CSF stimulation. Switching FDC-P1/MAC cells from growth in M-CSF to GM-CSF caused the selective degradation of c-*fms* mRNA within 6 h after factor switching. The degradation of c-*fms* mRNA could be blocked by both protein synthesis and transcriptional inhibitors.

Together, these data suggest that the trans-regulation of c-*fms* transcripts by GM-CSF requires the transcriptional activation of a selective mRNA degradation factor. In addition, preliminary evidence suggests that the 3' untranslated region of the c-*fms* transcript may contain a specific sequence/signal necessary for trans-regulation. The regulatory role this mRNA region plays is currently under investigation. These results begin to provide a molecular basis for the actions of GM-CSF and multi-CSF that may be important in governing myeloid lineage restriction and development.

M 308 A NOVEL MECHANISM THAT NEGATIVELY REGULATES EXPRESSION OF THE β SUBUNITS OF THE IL-3, IL-5 AND GM-CSF RECEPTORS

Takahiko Hara and Atsushi Miyajima,
DNAX Research Institute, Palo Alto, CA 94304

AIC2A and AIC2B are two homologous mouse genes encoding the β subunits of the IL-3, IL-5 and GM-CSF receptors. The AIC2A protein binds IL-3 with low affinity and is the β subunit of a high affinity IL-3 receptor. Although the AIC2B protein does not bind any cytokines by itself, it has proven to be the β subunit of the GM-CSF and IL-5 receptors. We examined expression of the AIC2A and AIC2B on various hemopoietic cell lines and found that expression of both AIC2A and AIC2B proteins were diminished in an IL-4-dependent- but IL-3-nonresponsive variant cell line (MC/9.IL-4). This cell line was derived from an IL-3 dependent mast cell line, MC/9, which expresses the AIC2 proteins at high level. Nuclear run-off assays and S1 analyses indicated that the AIC2A and AIC2B genes were transcribed but the mRNAs were degraded rapidly by a dominant factor present in the MC/9.IL-4 cells. Using stable transfectants, the target sequence of this dominant suppression was mapped within the coding sequence of the AIC2 genes. As this negative regulator suppresses expression of β subunits of the IL-3, IL-5, and GM-CSF receptors, it has a potential to eliminate all the three receptors simultaneously.

M 309 HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR: A ROLE FOR RESIDUE 21 IN HIGH BUT NOT LOW AFFINITY BINDING,

Timothy R. Hercus, M. Frances Shannon, Mathew A. Vadas, Bronwyn Cambareri, Mara Dottore, Nicos A. Nicola[1], Meredith J. Layton[1] and Angel F. Lopez. Division of Human Immunology, Institute of Medical and Veterinary Science, Box 14 Rundle Mall Post Office, Adelaide, SA 5000 and [1]The Walter and Eliza Hall Institute, Parkville, Victoria 3050, Australia.

Human granulocyte-macrophage colony stimulating factor (hGM-CSF) is a 127 amino acid glycoprotein with multiple biological activities including stimulating the proliferation of haemopoietic progenitor cells and the stimulation of mature haemopoietic cell function.

We have used substitution mutagenesis to examine the functional role of the predicted first alpha helix of hGM-CSF in multiple biological and receptor binding assays. Saturation mutagenesis has revealed one residue (Glu^{21}) that is highly sensitive to substitution, particularly by basic amino acids which alter the charge at this position. Analysis of one such mutant, GM-CSF(Arg^{21}), revealed a 200 fold reduction in biological activity that correlated with an equally reduced ability to bind the high affinity GM-CSF receptor on neutrophils. However, this mutant is equipotent with wild type hGM-CSF in binding to the cloned, low affinity α chain of the hGM-CSF receptor expressed on COS cells or to the low affinity component of the GM-CSF receptors on monocytes.

These results show that residue 21 is critical for the hGM-CSF biological activities tested and for high affinity but not for low affinity GM-CSF receptor binding. Thus the biological activity of GM-CSF is mediated through the high affinity receptor. The implication for GM-CSF ligand/receptor interactions will be discussed.

M 310 SIGNAL TRANSDUCTION IN SUPPRESSION OF APOPTOSIS BY GM-CSF AND IL-3,

T. Hoang, D. Rajotte, P. Haddad, A. Haman and E. Cragoe, Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7

Apoptosis is a process of active cell death characterized by the cleavage of cellular DNA into oligonucleosomic fragments. Both GM-CSF and IL-3 suppress apoptosis in hemopoietic cells. We have therefore investigated the possibility that these two growth factors may suppress apoptosis through activation of common events in a growth factor dependent cell line, using two complementary approaches: a) functional assays or immunochemical identification of intracellular mediators, and b) inhibition of their function through the use of specific inhibitors. Our data indicate that the suppression of apoptosis by GM-CSF and IL-3 is dose-dependent, with half efficient concentrations that are in the range of the dissociation constants of the high affinity GM-CSF and IL-3 receptors, respectively. Both IL-3 and GM-CSF induce a translocation of protein kinase C (PKC) to the membrane, followed by a sustained alkalinization of the intracellular pH. The use of PKC inhibitors (staurosporine, H7 and sphingosine) or inhibitors of the Na^+/H^+ antiport (HMA, EIPA) reverts the suppression of apoptosis by IL-3 and GM-CSF. Conversely, TPA, a PKC activator, can efficiently substitute for IL-3 or GM-CSF in maintaining cell survival. The protective effect of TPA is abrogated by staurosporine, a PKC inhibitor, or EIPA, an inhibitor of the Na^+/H^+ antiport. Taken together, our data are consistent with the view that the binding of GM-CSF or IL-3 to their receptors results in the sequential activation of PKC and of the Na^+/H^+ antiport, resulting in suppression of apoptosis in hemopoietic cells.

M 311 MITOGENIC INTERACTIONS OF RETROVIRAL ENVELOPE GLYCOPROTEINS WITH MURINE ERYTHROPOIETIN RECEPTORS,

Maureen E. Hoatlin, Susan L. Kozak, Frank E. Ferro, Jr. and David Kabat, Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201-3098

The interaction of Friend viral gp55 and the murine erythropoietin receptor (EpoR) have been studied using ^{125}IEpo in crosslinking experiments as well as a sensitive immunoprecipitation - Western blot procedure. These techniques have revealed that the plasma membrane form of gp55, $gp55^P$, is physically complexed with the EpoR. Taken together with data demonstrating the mitogenic stimulation of EpoR by gp55 in a lymphoid factor-dependent cell line and comparison of pathogenic and nonpathogenic gp55 mutants, we suggest that it is the surface component $gp55^P$ that is involved in the mitogenic stimulation of EpoR. Additionally, mitogenic stimulation has been studied using a retrovirus that encodes erythropoietin. This virus not only confers factor independent growth upon an IL3 dependent cell line expressing EpoR, but causes a disease in mice that mimics Friend disease including control by murine genes that determine susceptibility to Friend viral erythroleukemia. Furthermore, we have recently discovered that a retrovirus encoding EpoR causes a disease in susceptible mice that is indistinguishable from Friend disease. Thus, different mechanisms for causing constitutive EpoR activation all lead to indistinguishable diseases.

M 312 IL-4 INHIBITS THE POSITIVE SIGNAL OF IL-3 AND IL-1 ON THY lo LIN- SCA+ MURINE PLURIPOTENT HEMOPOIETIC STEM CELLS Susan Hudak, Shelly Heimfeld and Donna Rennick Department of Immunology DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA

Our previous work has shown that Thy lo Lin- Sca+ pluripotent hemopoietic stem cells (PHSC) are unresponsive to individual colony stimulating factors and respond poorly to IL-3. We have examined the effects of various factor combinations and found that multiple growth factor signaling is required to initiate the proliferation and differentiation of these cells. IL-1 was among the factors found to significantly enhance the clonal growth of PHSC when used in combination with IL-3. Recently, we have examined the effect of IL-4 on the colony forming potential of PHSC. Although IL-4 showed no effect on the small number of colonies generated in response to IL-3, it consistently inhibited the high colony numbers elicited by IL-1 plus IL-3. To better understand the interactions of these cytokines , the addition of either IL-1 or IL-4 was delayed for brief periods of time within the first 24 hours of culture. It was determined that an optimal response to IL-1 plus IL-3 required the presence of IL-1 from time zero. A delay of 6 hours reduced colony numbers by half and a delay of 24 hours reduced colony numbers to the level of IL-3 alone. It was also found that the inhibitory actions of IL-4 on the dual signaling of IL-1 plus IL-3 occurred within the first 24 hours. These results suggest that IL-1 and IL-4 act directly at the level of the PHSC, mediating their diverse effects early in the initiation of IL-3-dependent cell division. Recent work by Kitamura et al. (Int. Imm. 3:571,1991) indicates that IL-1 induces expression of the IL-3 receptor beta subunit on an erythroleukemic cell line TF-1. Currently, we are investigating the possibility that IL-1 also regulates the beta subunit expression on PHSC and that IL-4 blocks this action of IL-1 thus preventing PHSC from attaining an IL-3 responsive state.

M 313 MACROPHAGE INFLAMMATORY PROTEIN-1 ALPHA (MIP-1α) IS A DIRECT BIDIRECTIONAL MODULATOR OF HEMATOPOIETIC CELL GROWTH: COMPARISON WITH TRANSFORMING GROWTH FACTOR-BETA (TGF-β), [1]J.R. Keller, [2]S.E.W. Jacobsen, [2]F.W. Ruscetti. [1]BCDP, PRI/DYNCORP, [2]BRMP-LMI, FCRDC, Frederick, MD 21702.

The proliferation and differentiation of hematopoietic stem cells (HSC) is controlled, in part, by a balance of positive and negative growth signals. It has previously been shown that both TGF-β and MIP-1α inhibit the proliferation of HSCs. Surprisingly, TGF-β enhances GM-CSF-induced HSC colony formation while, MIP-1α enhances both GM-CSF and M-CSF induced colony formation. The studies presented here explore the spectrum of activities of these two cytokines on different HSC populations and to determine whether these effects were direct or indirect. While TGF-β only enhanced GM-CSF-induced bone marrow cell (BMC) colony formation, MIP-1α enhanced GM-CSF, IL-3 and CSF-1-induced colony formation of unfractionated and purified progenitors, designated lineage negative (LIN-). Single cell assays demonstrated that MIP-1α directly enhanced IL-3- and GM-CSF-induced proliferation but had no effect on CSF-1-induced growth. In comparison, TGF-directly inhibited IL-3- and CSF-1-induced proliferation and enhanced GM-CSF-stimulated growth of isolated LIN- cells. Finally, regardless of the growth promoting stimulus, both MIP-1α and TGF-β inhibited the growth of isolated Thy-1-positive LIN- progenitor cells. While the effect of TGF-β on HSC growth closely correlates with CSF receptor modulation, this mechanism was not observed with MIP-1α. Thus, MIP-1α and TGF-β have overlapping but distinct bidirectional effects on HSC growth.

M 314 THE ROLE OF *MET* AND HGF IN HEMATOPOIESIS

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Hepatocyte growth factor (HGF) has recently been shown to be a ligand for *met*, which is a member of the tyrosine kinase receptor family[a]. We have investigated the role of this ligand receptor pair in hematopoiesis. Previously, *met* expression was observed in several murine myeloid leukemia lines[b]. With one of these lines, NFS-60, HGF stimulated ^{3}H-thymidine uptake 400% when added in synergy with IL-3 in the presence of serum, but inhibited ^{3}H-thymidine incorporation 70% when added in the absence of serum. HGF added alone in the presence of serum had no effect. Given the effect of HGF upon the NFS-60 line, which is representative of myeloid progenitors, we investigated the effect of HGF upon a lineage negative progenitor population of bone marrow cells. HGF in synergy with IL-3 or GM-CSF increased colony formation from lineage negative cells by 50-60%. The additional colonies contained macrophages and granulocytes. HGF thus resembles IL-6 in stimulating the proliferation of hepatocytes and synergizing with IL-3 to stimulate the proliferation of hematopoietic progenitors. Our results indicate a significant role for *met* and HGF in regulating the proliferation of cells of the myeloid lineage.

[a]D.P. Bottaro *et al.*, *Science* 251, 802-804 (1991).
[b]A. Iyer *et al.*, *Cell Growth and Diff.* 1, 87-95 (1990).

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M 315 **MURINE MONOCLONAL ANTIBODIES DEVELOPED AGAINST THE RECEPTOR FOR THE GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF),** Urte Kyas, Torsten Pietsch, Birgit Teichmann, Petra Beenken, and Karl Welte, Dept. of Pediatric Hematology/Oncology, Medical School Hannover, Konstanty-Gutschow-Str.8, 3000 Hannover 61, FRG

The granulocyte colony-stimulating factor (G-CSF) is known to support growth and differentiation of neutrophil progenitor cells. The murine and human G-CSF receptor gene have been recently cloned and biochemically characterized. The aim of this study was to develop monoclonal antibodies against the purified murine G-CSF receptor. The G-CSF receptor protein used for immunization of CB6/F, mice was purified from the murine myeloid leukemia cell line NFS-60 by G-CSF affinity chromatography. This cell line is proliferating in response to rhG-CSF and was used for screening of hybridomas. The monoclonal antibodies 3/5 and 7/19/19 (isotype IgG2b) were able to compete with ^{125}I-rhG-CSF for the binding to the G-CSF receptor on NFS-60 cells in a dose dependent way. These antibodies were also used to detect human and murine G-CSF receptor protein in Western-blot analysis using the APAAP method and radioimmunoprecipitation from ^{35}S-methionine labelled NFS-60 cells. We identified G-CSF receptors using immunofluorescence and APAAP staining methods on human neutrophils, AML-blasts and IL-1 stimulated human endothelial cells. The specifity of the anti G-CSF receptor antibodies was documented by a negative control antibody (OKT3) and the inability of anti G-CSF receptor antibodies to stain cells without G-CSF receptors (e.g. lymphocytes). From these data we conclude that we developed monoclonal antibodies recognizing the G-CSF receptor on murine and human cells.

M 316 CHARACTERIZATION OF A NOVEL GENE EXPRESSED DURING GM-CSF STIMULATED MYELOPOIESIS, Elaine Y. Lin, Amos Orlofsky, Mark S. Berger, and Michael B. Prystowsky, Dept. of Pathology, University of Pennsylvania, Philadelphia, PA 19104

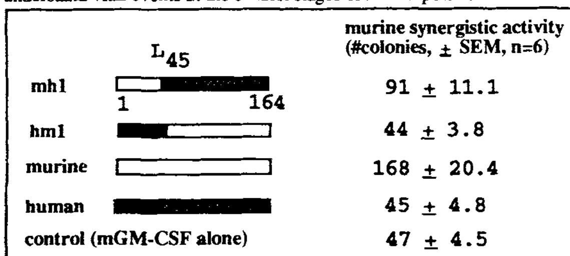
As part of our investigation of the regulation of gene expression during myelopoiesis, a cDNA clone of a novel murine gene (A1) has been identified. Northern analysis demonstrated that A1 gene expression was specifically induced in hemopoietic tissues, including bone marrow, spleen, and thymus. During GM-CSF stimulated myelopoiesis in cultured bone marrow cells, the gene expression was induced >10-fold within 24 hours. The A1 message also gradually accumulated during G-CSF stimulated differentiation of a myeloid progenitor cell line, 32D cl3. In addition, A1 gene expression was also induced >4-fold within 4 hours after LPS stimulation of the macrophage cell line, Raw 264.7. We have identified the complete A1 mRNA sequence and the size of the A1 protein predicted from the RNA sequence is about 20 kD. We speculate that A1 gene product may play a role(s) in hemopoietic cell development and cell activation.

M 317 MINIMAL TRANSCRIPTIONAL ELEMENTS REQUIRED FOR ERYTHROID GENE ACTIVATION BY EPO. KJ Lynch, D Taxman, and DM Wojchowski. Molecular & Cell Biology, The Pennsylvania State University, University Park, PA 16802

Primary regulation of terminal differentiation in red cells is exerted naturally by the hematopoietic glycopeptide, erythropoietin (EPO). Previous studies of induced pathways of erythroid gene activation typically have used erythroid MEL or K562 cell lines as in vitro models. These cells hemoglobinize in response to chemical inducers, but do not respond to EPO. In order to identify cis- and trans-factors which mediate authentic pathways of induced erythroid gene transcription, we have initiated studies of globin promoter activities in EPO-responsive J2E-1 cells. Preliminarily this involved the development of a modified transferrin/poly-L-lysine- mediated system for the efficient transfection of reporter constructs into J2E1 cells. Using this optimized system, activities of various natural and synthetic promoter constructs have been tested in induced vs. uninduced cells. Footprinting and transcriptional analyses indicate that a single GATA-1 element placed immediately upstream of the proximal -106 region of the murine B^{maj} promoter is sufficient to confer EPO-inducibility. Levels of activity for this construct are greater than (or equal to) the activity of the natural -300 promoter (+/- active domains from the human LCR), while the -106 promoter per se is relatively inactive. Current work aims to further delineate the absolute minimal set(s) of cis-elements which mediate EPO-induced transcription, and to identify a possible central role for GATA-1 in this pathway. [Supported by grants DK40242 and HL44491 (DMW) and F2-DK08688 (KJL)].

M 318 USE OF INTERSPECIES CHIMERIC POLYPEPTIDES TO STUDY THE STRUCTURE-FUNCTION RELATIONSHIPS OF STEM CELL FACTOR (SCF). Jeffrey V. Matous, Alex R. Cudkowicz, Virginia C. Broudy, and Kenneth Kaushansky. Division of Hematology, University of Washington, Seattle, WA 98195.

Stem cell factor (SCF, kit ligand, mast cell growth factor) is a growth factor important in the early development of hematopoietic, gonadal, and pigment stem cells. In order to study the structure-function relationships of the soluble form of SCF (the amino terminal 164 amino acids of the extracellular domain) we have exploited the finding that while both murine SCF (mSCF) and human SCF (hSCF) will stimulate growth in human marrow colony assays, only mSCF will do so in murine colony assays. As hSCF and mSCF share 79% sequence identity (86% if conserved amino acid substitutions are included) the two proteins likely fold in very similar ways. On the basis of these findings, we have generated interspecies hybrid SCF cDNA, expressed recombinant proteins in BHK cells, and tested the conditioned culture medium for the capacity to act synergistically with mGM-CSF in a murine marrow assay. As depicted in the figure, the SCF chimera mh1 retains mouse-specific synergistic activity. In contrast, hm1 was devoid of activity in this assay. These findings suggest that the amino terminal 45 residues are critical for the species-specific activity of SCF. Thus, the inability of the hm1 construct to stimulate murine marrow progenitor cells is likely due to the loss of critical ligand-receptor interactive residues. Further hybrids and site-specific mutants more specifically examining the residues within and just beyond this amino-terminal region are underway. Ultimately, we hope to characterize and define the structure-function relationships of SCF and thereby better understand vital events in the earliest stages of hematopoiesis.



M 319 LIPOXYGENASE METABOLISM IS REQUIRED FOR INTERLEUKIN-3 DEPENDENT PROLIFERATION AND CELL CYCLE PROGRESSION OF THE HUMAN M-07e CELL LINE, Miller A.M., Kobb S.M., and Turk, A. University of Florida, College of Medicine, and the Veterans Affairs Medical Center, Gainesville, Fl. 32610

The cell line M-07e requires the presence of either Interleukin-3 (IL-3) or Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) for continued proliferation in vitro. Cells deprived of growth factor for up to 48 hours remain viable, but no longer divide. On reexposure to IL-3 the growth factor deprived M-07e cells begin to divide within 48 hours. Using flow cytometric analysis of M-07e cells labelled with hypotonic propidium iodide we have demonstrated that the percentage of cells undergoing DNA synthesis decreases from 24%, in a log phase population of IL-3 stimulated cells to 1% after 24 hours of IL-3 deprivation. IL-3 deprived cells accumulate predominantly in a flow cytometry peak representative of G0/G1. DNA synthetic activity as determined by tritiated thymidine uptake and flow cytometry resumes between 12 and 18 hours after reexposure to IL-3, reaching a peak of up to 40% by 24 hours and returning to log phase levels by 72 hours. When IL-3 is added in the presence of any of three lipoxygenase inhibitors tested (Piriprost, caffeic acid, nordihydroguiaretic acid) there is a dose dependent inhibition of the resumption of proliferation and of DNA synthesis. Flow cytometry demonstrates that the cells remain in the G0/G1 population and do not progress through the cell cycle. These results are consistent with our previous observation that an intact lipoxygenase pathway is necessary for hematopoietic growth factor stimulated colony formation of normal bone marrow myeloid progenitors, and suggest that the induction of a lipoxygenase metabolite or metabolites is necessary for myeloid cells to progress through the cell cycle when stimulated by a hematopoietic growth factor.

M 320 EXPRESSION OF THE IMMUNOSUPPRESSIVE MOLECULE PLACENTAL PROTEIN 14 (PP14) BY THE MYELOGENOUS LEUKEMIC CELL LINE K562. Dwight M. Morrow, Robert R. Getty, L. Riittinen*, M. Seppala* and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106. * First Department of Obstetrics and Gynecology, Helsinki University Central Hospital, SF-00170 Helsinki, Finland.
Placental protein 14 (PP14) is a 28kD glycoprotein secreted by endometrial tissue during the first and second trimesters of pregnancy. PP14 is also present in high concentrations in the seminal fluid of men. Previous functional studies have demonstrated that PP14 isolated from extracts of decidual tissue can completely abrogate proliferation of lymphocytes in mixed lymphocyte reactions (MLRs). Since a developing fetus is essentially a semi-allograph expressing male specific antigens it could, in principal, be rejected by the mother. It has been suggested that PP14 expression may function to inhibit the mother's immune system and thus prevent fetal rejection.

In the course of differentially screening a cDNA library from the human myelogenous leukemia cell line K562 we unexpectedly identified a differential cDNA corresponding to PP14. Expression of PP14 mRNA by this leukemic cell line was further corroborated by northern blot analysis. Immunoprecipitation of supernatants from metabolically labeled K562 with anti-PP14 antibodies demonstrated a single protein band migrating at 28kD consistent with PP14's published size. These supernatants contained a potent antiproliferative activity when added to mixed lymphocyte cultures (MLR). This suppressive activity was completely reversed when antibody, which specifically immunoprecipitated PP14, was added to the MLR cultures.

This is the first demonstration of PP14 expression by a transformed leukemic cell line and suggests a possible mechanism whereby a leukemic cell could inhibit an anti-tumor immune response. Studies are in progress to ascertain if PP14 is expressed by non-transformed hematopoietic cells.

M 321 IDENTIFICATION OF THE MURINE INTERLEUKIN-3 RECEPTOR ASSOCIATED TYROSINE KINASE. Alice Mui*, Tony Pawson**, Gerald Krystal*, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, British Columbia, Canada*, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada**
Murine interleukin-3 (mIL-3) is a potent hemopoietic growth factor that is produced by activated T lymphocytes and stimulates the proliferation and differentiation of pluripotent stem cells and committed myeloid and early lymphoid progenitors. On addition to mIL-3 dependent cell lines it very rapidly stimulates the tyrosine phosphorylation of several proteins, including the 140 kD receptor itself. Recent cloning of this receptor, however, indicates that it does not contain a predicted tyrosine kinase domain and thus its tyrosine phosphorylation must be mediated by a distinct protein. Studies in our laboratory with an mIL-3 dependent cell line, $B6SUtA_1$, that expresses high levels of mIL-3 receptors (i.e. 100,000/cell) have revealed that, apart from the mIL-3 receptor, four proteins become rapidly tyrosine phosphorylated. Since all tyrosine kinases described to date autophosphorylate upon activation, candidates for the mIL-3R tyrosine kinase include these four proteins which have apparent molecular weights of 95 kD, 70 kD, 55 kD and 32 kD. Intriguingly, the phosphorylation of the mIL-3R and these four proteins in response to mIL-3 is maximal by 2 minutes even at 4°C, suggesting that these four proteins must be closely associated with the mIL-3R. Western analyses rule out fps/fes and hck as one of these proteins. Interestingly, the tyrosine phosphorylated 95 kD protein co-immunoprecipitates with anti-GAP (anti-Ras GTPase activating protein) and binds the SH2 (src homology 2) domain of GAP. Since GAP associates with tyrosine kinases, this mIL-3 activated 95 kD protein is a good candidate for the mIL-3R tyrosine kinase. We are currently utilizing anti-phosphotyrosine and SH2 affinity chromatography to purify this protein. An analysis of its sequence should allow assessment of this possibility.

M 322 EVALUATION OF THE ABILITY OF MAST CELL GROWTH FACTOR TO ENHANCE RECOVERY FROM RADIATION-INDUCED HEMOPOIETIC APLASIA. M.L. Patchen*, R. Fischer*, D.E. Williams+, and T.J. MacVittie*, *Armed Forces Radiobiology Research Institute, Bethesda, MD and +Immunex Corporation, Seattle, WA.
Based on *in vitro* studies, the *c-kit* ligand mast cell growth factor, MGF, has been implicated as an important hemopoietic regulatory factor, especially in the presence of additional hemopoietic cytokines. We have evaluated the ability of MGF administration to alter hemopoiesis *in vivo* using a model in which B6D2F1 mice exposed to a sublethal 7.75 Gy dose of cobalt-60 gamma radiation exhibit a temporary 14-17 day reduction in bone marrow and splenic granulocyte-macrophage progenitor cells (GM-CFC) and spleen colony-forming units (CFU-s), followed by recovery to normal levels. Recovery in this model is presumed to be mediated by endogenously produced hemopoietic cytokines. We hypothesized that the administration of MGF to such mice may synergize with endogenous cytokines and accelerate hemopoietic recovery. Female B6D2F1 mice were irradiated, administered either saline or recombinant murine (rm) MGF (50, 100, 200, or 400 μg/kg, s.c.) daily, and on days 14 and 17 postexposure assayed for bone marrow and splenic GM-CFC and CFU-s recovery, as well as peripheral blood white cell (WBC), red cell (RBC) and platelet (PLT) recovery. While radiation control mice and mice receiving 50, 100, and 200 μg/kg/d of rmMGF survived to day 17 postirradiation, approximately 30% of mice receiving 400 μg/kg/d died prior to day 17. A dose related increase in bone marrow and splenic GM-CFC, splenic CFU-s, and peripheral WBC, RBC, and PLT recoveries was evident following 17 days of rmMGF administration. Maximum responses were observed at 200 μg/kg/d. In these mice, bone marrow and splenic GM-CFC recoveries were 250% and 567% of radiation control recoveries, splenic CFU-s recovery 175% of radiation control recovery, and peripheral blood WBC, RBC, and PLT recoveries 164%, 122%, and 281% of radiation control recoveries. These studies illustrate the ability of rmMGF to affect multilineage hemopoietic regeneration *in vivo* following radiation-induced hemopoietic aplasia.

M 323 IN VITRO AND IN VIVO HEMATOPOIETIC ACTIVITY OF A NOVEL SYNTHETIC HEMATOREGULATORY PEPTIDE. Louis M. Pelus, Peter DeMarsh, Andrew King, Joanna Balcarek, Carrie Frey, Pradip Bhatnagar, Robert Levin, Robert Scott. Depts. of Anti-Infectives, Peptidomimetic Research and Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406
We have replaced the cysteine bridge of hematoregulatory peptide HP5 $(pEEDCK)_2$ with a nonreducible dimethylene carbon bridge $(pEEDSubK)_2$ creating a new peptide SK&F107647. This peptide has been evaluated for its myelopoietic and anti-infective effects in vitro and in vivo. A human CSA producing marrow derived fibroblastic stromal cell line (C6) was established and used to analyze the effects of this peptide on CSA production quantitated on murine CFU-GM. Addition of cell free supernates from C6 cells pulsed for 1 hour with peptide resulted in increased CSA production reaching half-maximal levels at a peptide concentration of 1-5 pg/ml. The CSA detected could be neutralized with a monoclonal antibody to M-CSF. Northern analysis indicated a rapid short term upregulation of M-CSF mRNA detectable within 15 minutes and reaching maximum at 30-60 minutes post peptide treatment. Single injection of 1-10 ng/kg of peptide in intact normal mice resulted in a 4-6 fold increase in serum CSA levels reaching maximum at 6 hours post injection. Administration of 0.1 to 1000 ng/kg SK&F107647 QDx4 results in stimulation of the proportion of CFU-GM in S-phase (36±6 vs 77±3, P<0.005 n=6 expts). A >50% increase in total CFU-GM/femur and a doubling of day 8 CFU-S (5.8±.4 vs 10.8±1.2 per $2x10^4$ marrow cells; P<0.01, n=8) were also observed. The effects of SK&F107647 on CFU-GM cell cycle rate and absolute number were identical to that observed following QDx4 administration of 20,000 U/mouse of recombinant human M-CSF. Administration of 10 ng/kg SK&F107647 QDx7 results in a 150-200% increase (P<0.05, n=3) in peritoneal macrophage superoxide production and significant Candidacidal activity (25-60% CFU reduction; P<0.01, n=3) which correlates with significant protection from lethal challenge with C. albicans in normal and immunosuppressed (3 Gy) BALB/c mice. A sc continuous infusion model in femorally cannulated Fisher rats was utilized to evaluate the effects of peptide on peripheral blood WBC and differentials. Animals received 1, 10 or 100 ng/kg/day of peptide delivered by a sc implanted 14 day Alzet pump. Serial blood samples were obtained by withdrawal from the cannula maintained with heparinized dextrose. Significant elevation of PMN, monocytes and platelets were observed at a dose of 1 ng/kg within 4 days with maximal increases observed by days 7-11. SK&F107647 represents a novel compound which shares biological and anti-infective activity with hematopoietic growth factors.

M 324 MITOGENIC EFFECTS OF HUMAN STEM CELL FACTOR (*c-kit* LIGAND) ON SCF-RECEPTOR POSITIVE MYELOID LEUKEMIA CELLS: HETEROGENEITY IN RESPONSE AND SYNERGY WITH OTHER HEMATOPOIETIC GROWTH FACTORS, Torsten Pietsch, Urte Kyas, Uwe Steffens, Martin R. Hadam, Wolf-Dieter Ludwig, Hans Drexler, Krisztina Zsebo and Karl Welte, Department of Pediatric Hematology and Oncology, Medical School Hannover, Department of Medicine, Klinikum Steglitz, Free University of Berlin, German Collection of Microorganisms and Cell Cultures, Braunschweig, FRG, and Amgen, Thousand Oaks, CA

We studied the mitogenic activity of recombinant human stem cell factor (rhSCF) on myeloid leukemia cells which expressed the SCF receptor (SCF-R, c-kit-protein). Using ^{3}H-thymidine uptake assays, proliferation of 12 SCF-R^{+} myeloid leukemia cell lines as well as fresh myeloid leukemic blasts from 20 patients with SCF-R^{+} AML was tested in the presence of various concentrations of rhSCF alone or in combination with saturating concentrations of rhG-CSF, rhGM-CSF or rhIL-3. Five out of 12 lines, and fresh leukemic blasts from 13 of 20 AML patients significantly responded to rhSCF alone. The stimulation indices ranged between 2 and 235. The responding cell lines were of the acute promyelocytic, chronic myeloid, megakaryoblastic and erythroleukemic origin, the responding fresh leukemic blast preparations of various FAB subtypes. Synergistic activities of rhSCF was seen when rhSCF was combined with rhG-CSF, rhGM-CSF or rhIL-3 in a subgroup of the SCF-R^{+} leukemias. To determine the SCF binding sites on leukemic cells, we used ^{125}I-rhSCF in Scatchard analysis and crosslinking studies. The leukemic cell lines expressed between 1200 and 29,000 high affinity binding sites per cell. Crosslinking studies demonstrated a 150 kD SCF receptor on the surface of all SCF-R^{+} myeloid leukemias. The proliferation in response to rhSCF did not correlate with the SCF receptor numbers expressed on the cell surface. This study suggests that SCF may be an important factor for the growth of myeloid leukemia cells, either as a direct stimulus or as a synergistic factor for other cytokines. In addition, using PCR analysis of mRNA we found expression of SCF-mRNA in 6 of the 12 myeloid leukemia lines, suggesting autocrine growth mechanisms by coexpression of SCF and its receptor.

M 325 BIOSYNTHETIC AND ENDOCYTOTIC PROPERTIES OF EPO RECEPTORS MUTATED AT WSXWSE AND CYTOSOLIC DOMAINS, D.E. Quelle and D.M. Wojchowski, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802

Erythropoiesis is promoted strongly through the interaction between the glycopeptide hormone erythropoietin (EPO) and its cloned cell surface receptor. This receptor, and a growing number of non-PTK receptors for alternate cytokines, share homology within extracellular domains, including a well-conserved WSXWSE motif. To explore the significance of this motif, five WSXWSE EPO receptor mutants were prepared and their activity, biosynthetic processing, and endocytotic properties were compared. Receptors mutated as Trp^{235}->Gly, Trp^{235}->Phe, or Trp^{232}->Gly and Trp^{235}->Gly failed to transduce EPO-induced growth, while Ser^{236}->Thr and Glu^{237}->Lys mutants exhibited partial activity (50-75% of wild type). Inactive forms also showed altered patterns of glycosylation with an increased sensitivity to Endoglycosidase H, thus suggesting a reduced efficiency in transport to the plasma membrane. In contrast, internalization assays (performed using a novel ^{32}P-labeled EPO) showed that all mutants specifically bound and internalized ligand, indicating that an intact WSXWSE motif is not required for these events. Interestingly, forms of receptor with nearly fully deleted cytosolic domains (e.g., 219 of 226 COOH-terminal AA; inactive form) also specifically internalized EPO. Together, these findings suggest that while the WSXWSE domain apparently is important for EPO receptor processing and growth signal transduction, neither this, nor any cytosolic domains mediate ligand internalization. (Supported by NIH grants R29-DK40242 and HL44491).

M 326 IL-3, GM-CSF, AND TRANSFECTED ERTHROPOIETIN RECEPTORS MEDIATE TYROSINE PHOSPHORYLATION OF A COMMON CYTOSOLIC PROTEIN (pp100) IN FDC-ER CELLS, Frederick W. Quelle, Dawn E. Quelle and Don M. Wojchowski, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802

The hematopoietic growth factor, erythropoietin, is a prime regulator of red blood cell production in mammals. Towards defining cellular events which are associated with the proliferative action of this factor, we have studied induced protein phosphorylation in murine myeloid FDC-P1 cells stably transfected with an erythropoietin receptor cDNA (FDC-ER cells). Unlike parent FDC-P1 cells, FDC-ER cells proliferate in response to erythropoietin (Quelle, D.E., and Wojchowski, D.M. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 4801-4805), and presently are shown to rapidly phosphorylate a Mr 100,000 cytosolic protein (pp100) at tyrosine residues in response to this factor. Furthermore, tyrosine phosphorylation of pp100 also is shown to be induced in FDC-ER and FDC-P1 cells in response to IL-3 and GM-CSF. However, limited differences in the phosphopeptide maps of pp100 as isolated following exposure to erythropoietin versus IL-3 were observed, suggesting that IL-3 may induce phosphorylation of pp100 at additional sites. Also, as compared to parent FDC-P1 cells, a selective loss in FDC-ER cells of proliferative responsiveness to GM-CSF was associated with a reduced capacity of GM-CSF to induce the phosphorylation of pp100. These findings establish a role for the murine erythropoietin receptor in mediating protein tyrosine phosphorylation during erythropoietin-induced growth, and are consistent with a role for pp100 as a common transducer in the apparently convergent pathways of erythropoietin-, IL-3-, and GM-CSF-induced proliferation of myeloid progenitor cells.

M 327 STRUCTURE-FUNCTION ANALYSIS OF THE HUMAN GM-CSF RECEPTOR, Lucienne Ronco, Sheryl L. Silverman, and Judith C. Gasson, Departments of Biological Chemistry and Medicine (Hematology-Oncology), UCLA School of Medicine, Los Angeles CA

GM-CSF stimulates the proliferation of myeloid progenitor cells and enhances the functional activities of mature granulocytes and monocytes. GM-CSF interacts specifically with both hematopoetic and nonhematopoetic cells via a membrane-bound receptor complex. Two of the receptor molecules necessary for this interaction have recently been cloned (alpha and beta subunits), and were found to be members of a newly described growth factor and cytokine receptor superfamily (Gearing et al., *EMBO J* 8:3667-3676, 1989; Hayashida et al., *PNAS* 87:9655-9659, 1990). Characteristics of the superfamily include four spatially conserved cysteine residues, a serine-tryptophan motif in the extracellular domain, and a proline-serine-rich cytoplasmic domain. Mutational analyses of the alpha subunit of the GM-CSF receptor have been undertaken to delineate regions responsible for ligand binding and internalization. Future experiments will focus on the ability of mutant alpha subunit constructs to associate with the beta subunit and transduce a proliferative signal.

M 328 THE HUMAN IL4 RECEPTOR SHARES A CRITICAL SIGNAL TRANSDUCTION DOMAIN WITH OTHER CYTOKINE RECEPTORS, David C. Seldin and Philip Leder, Dept. of Genetics, Harvard Medical School, Boston MA 02115.

The receptors for interleukins 2, 3, 4, 5, 6, 7, GM-CSF, G-CSF, EPO, LIF, GH, PRL, and ciliary neurotropic factor form a "cytokine receptor superfamily" defined by the presence of extracellular paired cysteines and a WSXWS motif. The structure of the cytoplasmic domains of these receptors appears to be less well-conserved and no known elements responsible for signal transduction have been identified. Known "second chains" of these receptors are in the same family and have not illuminated the mechanism of signal transduction. We showed that signal transduction elements of the IL4 receptor are conserved across species and cell types by demonstrating that mouse myeloid (FDCP1) and lymphoid (BaF3) cells would proliferate in human IL4 when stably transfected with the HIL4R. Mutant receptors were made by restriction digestion and by recombinant PCR and expressed in BaF3 cells. mRNA expression was determined by ribonuclease protection and protein expression was determined by binding of an HIL4-alkaline phosphatase fusion protein and flow cytometry. Analysis of these mutants shows that two-thirds of the C-terminal portion of the cytoplasmic domain can be deleted without any effect on signal transduction in BaF3 cells. Deletion of additional amino acids abrogates signal transduction. The critical domain includes a 23 amino acid sequence which is moderately conserved between the critical signal transduction domains defined for IL2Rβ, EPOR, G-CSFR, and IL4R. Studies are underway to determine whether this domain in isolation can transduce a signal. Additional mutants will be used to investigate the mechanism of signal transduction.

M 329 CANINE STEM CELL FACTOR (C-KIT LIGAND) SUPPORTS THE SURVIVAL OF HEMATOPOIETIC PROGENITORS IN LONG-TERM MARROW CULTURE, Robert M. Shull[1], Sidney V. Suggs[2], Keith E. Langley[2], Kenneth H. Okino[2], Frederick W. Jacobsen[2], and Francis H. Martin[2], [1]Department of Pathobiology, The University of Tennessee College of Veterinary Medicine, Knoxville, TN 37996 and [2]AMGEN, Inc., Thousand Oaks, CA 91320.

The cDNA for canine stem cell factor (cSCF, c-kit ligand) was cloned and expressed in *Escherichia coli*. The recombinant protein (rcSCF), 165 amino acids in length, is very similar structurally to the soluble form of previously cloned and sequenced rodent and human SCF's. The biological effects of rcSCF were studied in a day 10 CFU-GM clonogenic assay and in long-term liquid bone marrow culture of nonadherent hematopoietic cells in the absence of a stromal underlayer. Synergism in the stimulation of growth of day 10 CFU-GM was demonstrated between rcSCF and both recombinant human (rh) GM-CSF and naturally occurring colony stimulating activity present in the serum of a neutropenic dog. Alone, rcSCF was nonstimulatory for committed marrow precursors in methylcellulose cultures and had minimal effect on hematopoietic stem cell survival in stromaless liquid cultures. When rcSCF was combined with rhIL-6 or phytohemagglutinin-stimulated canine lymphocyte-conditioned medium, culturable stem cells survived for 3-5 weeks. Stem cell survival was negligible beyond one week when cultures contained other growth factor combinations, but lacked rcSCF. When combined with rhGM-CSF, with or without rhIL-6, rcSCF led to a marked increase in day 10 CFU-GM which was not sustained beyond one week in liquid culture. It appears that rcSCF, like that from rodent and primate species, has the ability to influence the survival and proliferation of committed and earlier progenitor cells in hematopoietic tissues. In a long-term liquid culture system where growth factor production by stromal cells is essentially eliminated, rcSCF possesses a unique ability to maintain the viability of progenitor cells for up to 5 weeks.

M 330 THE EFFECT OF LOW MOLECULAR WEIGHT B CELL GROWTH FACTOR ON CELLS FROM PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA, Cecilia Skjønsberg, Leiv Rusten, Surendra Sharma* and Heidi Kiil Blomhoff, Laboratory of Immunology, Institute for Cancer Research, The Norwegian Radium Hospital, 0310 Oslo, Norway and * Department of Pathology, Roger Williams General Hospital, Brown University, Providence, U.S.A.

Low molecular weight B cell growth factor (BCGF), a 12kD cytokine, plays a significant role in proliferation of cells from the B lineage. To our knowledge, BCGF has hitherto not been shown to have any effect on myeloid cells. We have examined the effect of BCGF on cells from patients with acute myeloblastic leukemia. Leukemic cells from 20 patients were highly purified by removing contaminating B- and T-cells by rosetting with immunomagnetic beads. LMW-BCGF induced significant increases in cell proliferation in cells from 18 of 20 patients as measured by DNA-synthesis and colony formation in methylcellulose. Cell cycle analysis showed that LMW-BCGF promoted S-phase entry of the cells. There is no evidence for any differentiation of the cells as judged by uptake of nitroblue tetrazolium dye and morphology. LMW-BCGF is known to contain various concentrations of other cytokines such as TNFα, TNFβ and IL-2. To ensure that the reported effects of LMW BCGF were true BCGF effects, human recombinant BCGF was used as a control, with similar results obtained. Taken together, our results show that BCGF has effects on myeloid cells, yet another example of the pleiotrophic effects of cytokines.

M 331 DISREGULATION OF THE EXPRESSION OF M-CSF RECEPTOR AND PDGF RECEPTOR GENES DURING F-MuLV-INDUCED MYELOBLASTIC LEUKEMOGENESIS.

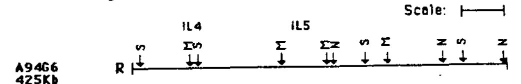
Brigitte Sola, Nathalie de Parseval, Serge Fichelson, Patrick Mayeux and Sylvie Gisselbrecht. ICGM, INSERM U 152, Hôpital Cochin, 27 rue du Faubourg Saint-Jacques, 75014 Paris, FRANCE.

The slow-transforming Friend murine leukemia Virus (F-MuLV) induces myeloblastic leukemias in susceptible mice. In clonal myeloblastic cells, proviruses are integrated in three preferential sites named Fim-1, Fim-2 and Fim-3. The 15kb-long Fim-2 region is located upstream to the first coding exon of the c-fms proto-oncogene which encodes the macrophage colony-stimulating factor receptor (M-CSF R). Proviral integration in the Fim-2 region occurs in 30% of the leukemias tested and results in the overexpression of the c-fms mRNA. In these cells, the M-CSF receptor is normaly expressed at the cell surface, as M-CSF binding sites and MCSF addition gives a proliferative signal to the cell. Since myeloblastic leukemic cells also synthetize M-CSF, it is likely that this autocrine loop favors the transformation process.

The M-CSF receptor gene is tandemly linked to the platelet-derived growth factor ß receptor (ßPDGF R) gene and the two genes are in a very closed proximity. The ßPDGF R gene which is normaly expressed in epithelial cells, is transcribed as a normal sized mRNA in a high percentage of myeloblasic leukemias (7/21). ßPDGF binding sites are present at the cell surface of myeloblatic cells and able to bind the PDGF-BB with a high affinity. PDGF-BB also tranduces a proliferative signal in these cells. However few receptor molecules are expressed and we are currently testing for the presence of PDGF-BB ligand in the cells. Interestingly, the c-fms and the ßPDGF receptor genes are independently expressed in myeloblastic leukemias. In fact, the expression of the ßPDGF receptor gene is not due to a proviral integration in the Fim-2 region and we are studying the mechanism of this gene activation in these cells.

M 332 REGULATION OF G-CSFR mRNA EXPRESSION IN NORMAL AND LEUKEMIC CELL LINES, Richard A. Steinman and David J. Tweardy, Departments of Medicine and Molecular Genetics and Biochemistry, U. of Pittsburgh School of Medicine and the Pittsburgh Cancer Institute, Pittsburgh, Pa. 15213. Our laboratory is investigating the normal G-CSF differentiation pathway, using the nontumorigenic murine IL-3 dependent cell line clone 32Dcl3 as a model of granulocyte differentiation. We find that steady state expression of G-CSF Receptor (G-CSFR) mRNA in 32Dcl3 is increased in an immediate early response to its ligand. Similar upregulation occurs in murine bone marrow. Actinomycin D chase experiments indicate that this does not result from enhancement of G-CSFR mRNA stability by G-CSF. Cycloheximide superinduces G-CSFR message and also augments G-CSF-induction of G-CSFR. An excess of IL3 does not alter basal expression of receptor message in 32D, but does dampen the response to G-CSF. A survey of leukemic cell lines indicates that basal expression of G-CSFR, upregulation with G-CSF, and superinduction by cycloheximide is maintained in NFS60 and in ts-abl-transformed 32Dcl3 at the nonpermissive temperature. Constitutive expression of G-CSFR mRNA by WEHI-3B D+ cannot be modulated by ligand or cycloheximide. WEHI-3B D- cells and ts-abl transformed 32Dcl3 at the permissive temperature exhibit detectable message only in the presence of cycloheximide; upregulation by ligand can then be detected. In contrast, basal suppression of G-CSFR message in six v-abl transformed 32Dcl3 cell clones cannot be released by ligand or cycloheximide. These results suggest that leukemic transformation mediated by autocrine IL-3 production or v-abl may be associated with abnormal regulation of G-CSFR mRNA expression.

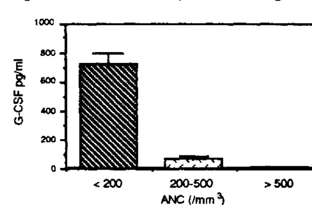
M 333 CHARACTERIZATION OF THE INTERLEUKIN GENE CLUSTER AT 5q31. W. Stock, W.L. Neuman, M.M. LeBeau, and C.A. Westbrook, Section Hematology/Oncology, University of Chicago, Chicago IL. 60637
Human *CSF2* (*GMCSF*) and interleukins *IL3*, *IL4*, and *IL5* have been localized to band 5q31 within the cluster of hematopoietic growth factors on the long arm of chromosome 5. We have previously reported the long-range physical mapping results of this region using Yeast Artificial Chromosome (YAC) clones, one containing *IL3* and *CSF2*, and one containing *IL4* and *IL5*. We confirm the close linkage of *IL3* and *CSF2* (within 8 kb) and show that *IL4* and *IL5* are widely separated (100-175Kb) and do not overlap with the *IL3/CSF2*-containing YAC. The fine resolution map of the *Not*I (N), *Sfi*I (S), and *Mlu*I (M) sites surrounding the *IL4* and *IL5* genes is shown below:



We postulate that the large genomic regions surrounding the *IL4* and *IL5* genes may contain other genes involved in hematopoiesis. To investigate this possibility, we have prepared phage libraries from each YAC and have isolated phage clones containing N, S, and M sites. These rare-cutting restriction endonucleases identify GC-rich genomic regions and can be used to identify the location of new genes. We are currently screening these phage clones for the presence of phylogenetically conserved sequences (using zoo blots) which could identify the presence of a new gene. Also, to determine the order of the known hematopoietic growth factors on 5q31, additional phage clones containing *IL3*, *CSF2*, *IL4*, *IL5*, and the YAC end fragments have been isolated and are being ordered using dual-color fluorescent in situ hybridization (FISH).

M 334 EVIDENCE FOR COMMON REGULATORY PATHWAYS AND/OR SHARED SUBUNITS FOR SEVERAL MURINE HEMATOPOIETIC GROWTH FACTOR RECEPTORS, Carol Stocking, Jurgen Hannemann, Christine Laker, Jutta Friel, Gabriel Beck-Engeser, Atsushi Miyajima, and Wolfram Ostertag, Heinrich-Pette-Institut fur Immunologie und Virologie, Hamburg 20, D-2000 Germany and DNAX Research Institute, Palo Alto, CA 94304-1104, USA
We have isolated several sets of mutants from two myeloid progenitor cell lines, FDC-P1M and D35, that were selected on the basis of either growth factor independence or responsiveness to GM-CSF. Analysis of the factor independent mutants has shown that 2/9 spontaneous mutants and 5/62 mutants obtained after retroviral insertion mutagenesis exhibited either altered binding of GM-CSF and/or IL-3 or altered expression of one or more of the receptor subunits for these factors. Closer analysis of one of these mutants has revealed a rearrangement in the extracellular domain of the beta subunit of the GM-CSF receptor. Unexpectedly, this mutant has lost its binding capacity for both GM-CSF and IL-3. These results suggest that this subunit might facilitate both GM-CSF and IL-3 binding, a phenomenon observed in the human system, but not yet documented in the murine system. Analysis of 7 mutants obtained after retroviral insertional mutagenesis and selected on basis of responsiveness to GM-CSF has revealed that unlike the parental cell lines, these cells now exhibit high-affinity receptors for GM-CSF. Unexpectedly, however, these cells are now also responsive to SCF. Although transcripts for SCF receptor (*kit*) could be detected in the parental cell line, only mutants which have gained responsiveness to GM-CSF will proliferate in the presence of SCF. These results suggest a common regulatory or transduction mechanism for these two growth factors. Analysis of retroviral integration sites should help identify the mutation.

M 335 ENDOGENOUS G-CSF LEVELS AFTER ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION CORRELATE WITH MYELOID ENGRAFTMENT, Yu Suen, Eva Knoppel, Carmella van de Ven, Mitchell S. Cairo, Children's Hospital of Orange County, Orange, CA 92668
Myeloid engraftment following bone marrow transplantation (BMT) is influenced by a number of variables including genetic disparity, preparative therapy, BM harvest and purging regimen, and postgraft systemic complications. Granulocyte-colony stimulating factor (G-CSF) stimulates the proliferation of myeloid progenitor cells and enhances myeloid engraftment following BMT. We investigated the endogenous G-CSF levels and myeloid engraftment in both children and adults following allogeneic (ALLO) and autologous (AUTO) BMT. Serum G-CSF levels were measured by an Elisa assay using polyclonal rabbit anti-rhG-CSF-Ig-coated microtiter plates. Patient serum samples were incubated overnight. HRP conjugated mouse monoclonal anti-G-CSF antibody was added and TMB was used as a substrate to detect serum G-CSF levels. After BMT, G-CSF levels rose significantly during severe neutropenia (<200/mm^3) and then began to fall during resolution of neutropenia and beginning of myeloid engraftment. The correlation coefficient between the log of endogenous G-CSF levels and the ANC count was r= -0.87 (p<0.001). Both children and adults demonstrated a similar inverse relationship between the circulating G-CSF level and the degree of neutropenia. One patient failed to engraft following autologous BMT and additionally failed to generate any endogenous G-CSF production. Once the endogenous G-CSF level fell below 200 pg/ml, there was a mean of 6.0 ± 0.9 days until engraftment (ANC ≥500/mm^3). This study demonstrates that endogenous G-CSF production is associated with myeloid engraftment in both children and adults following AUTO and ALLO BMT and that the rate of rise and fall of circulating G-CSF levels may be predictive of the success of myeloid engraftment.



M 336 THE HUMAN IL5Rα CHAIN : PROPERTIES OF GENE AND PROTEIN, Jan Tavernier, Geert Plaetinck, José Van der Heyden and René Devos, Roche Research Gent, Jozef Plateaustraat 22, B-9000 Ghent, Belgium

cDNA clones and the genomic gene coding for the human IL5Rα chain have been analysed. The major transcript of this gene as detected in both HL-60 eosinophilic cells and eosinophilic myelocytes grown from cord blood, encodes a secreted form of this receptor. (Tavernier, J. et al., Cell 66, 1175-1184, 1991). This, and additional, variant transcripts of the hIL5Rα gene are very likely due to alternative splicing events. The genomic organization of the gene reveals a striking similarity with the human growth hormone receptor gene. This confirms the relationship of growth hormone to the interleukin/ haemopoietin family.

The exons of the hIL5Rα gene define a signal peptide domain followed by six domains, building up 3 repeated structural units of the extracellular part of the receptor. The soluble hIL5Rα isoform has antagonistic properties. A second component of the hIL5R complex has been identified as the β chain of the human granulocyte/ macrophage colony-stimulating factor (GM-CSF) high affinity receptor. This Rβ chain is in addition also part of the hIL3R complex. Evidence for a hierarchic nature of binding of the ligand-Rα -chain complexes to this shared Rβ chain will be presented.

M 337 ISOLATION AND CHARACTERIZATION OF THE CD11b MYELOID SPECIFIC PROMOTER, Daniel G. Tenen, Heike L. Pahl, Donna E. Becker, Consuelo M. Glenn, and Roger M. Perlmutter, Hematology-Oncology Division, Department of Medicine, and Department of Genetics, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215, and University of Washington, Seattle, WA 98195. CD11b is the alpha chain of the Mo1 adhesion heterodimer, which is specifically expressed in myeloid (granulocyte/monocyte) lineages and NK cells, but not in other cell types. CD11b mRNA is specifically expressed in myeloid cells and up-regulated in myeloid cell lines induced to differentiate in vitro; nuclear run-on experiments demonstrate that the up-regulation is predominantly transcriptional. We have isolated the 5' upstream CD11b genomic region including a 1.7 kb sequence which directs myeloid-specific transcription of a reporter luciferase gene in transfected HL-60 and U937 cells, but not in HeLa cells. Luciferase expression in these constructs is increased when the cells are induced to differentiate with the phorbol ester TPA. Deletion analysis localizes elements directing high levels of tissue specific reporter gene expression to the 414 bp proximal to the transcriptional start site. This sequence contains two consensus binding sites for Sp1, a GATA motif and a purine rich sequence, which presents potential binding sites for members of the ets family of genes, including Spi-1, a B cell/macrophage factor. A point mutation in the GATA box which abolishes binding of GATA-1 does not diminish CD11b promoter activity. Preliminary experiments indicate the binding of a Spi-1 like activity in the proximal promoter area. Functional characteristics of the CD11b promoter were tested in transgenic mice. Blood cells expressing endogenous CD11b and some B cells were found to express the transgene while most other cells, including T cells did not. Future experiments shall be directed at localizing the precise sequences responsible for myeloid specific expression, and attempting to identify and isolate myeloid specific transcription factors. Such factors may play a role in normal myeloid differentiation, and could potentially be involved in the pathogenesis of acute myelogenous leukemia.

M 338 DIFFERENTIAL ACTIONS OF RECOMBINANT IL-3 AND SCF IN A CLONED, FACTOR-DEPENDENT CELL LINE, Connie S. Tettenborn and Jeffrey N. Siegel, Immune Cell Biology, Naval Medical Research Institute, Bethesda, MD 20889

The product of the steel gene is now known to be stem cell factor (SCF) which has a profound influence on the development of several embryonic cell types, including those of the hematopoietic lineage. To investigate the mechanism of SCF action, we compared its effects with those of IL-3 in the megakaryoblast cell line, M-07E, which requires IL-3 for growth. We found that M-07E cells respond to IL-3 and SCF in different ways. While IL-3-deprived M-07E cells die rapidly, the addition of SCF to cells grown in 10% serum-containing medium enhances cell survival. Cells proliferate normally when retreated with IL-3 but not SCF, even at high doses. Interestingly, the combination of SCF and IL-3 resulted in synergistic proliferation, suggesting that their receptors are coupled to different signalling pathways. To define the molecular basis for the different actions of SCF and IL-3, we measured the expression of immediate-early response (I-ER) genes in growth factor-starved M-07E cells treated with SCF or IL-3. Since we plan to extend these studies to cell-sorted bone marrow, we used the sensitive technique of reverse transcriptase-polymerase chain reaction. The level of mRNA could be quantitated using this technique, since amplification of the housekeeping gene, GAPDH, was shown to be linear with respect to input RNA concentration. Several I-ER genes, such as myc, are known to be essential for proliferation. Preliminary results suggest that myc expression is induced by both IL-3 and SCF. Results with fos and jun will also be presented. In conclusion, IL-3 and SCF appear to have unique and synergistic effects on M-07E cell growth. Examination of I-ER gene expression should help to elucidate the basis for their different effects.

M 339 RESPONSE TO STEM CELL FACTOR (SCF) AND COLONY-STIMULATING FACTORS (CSF) AND DIFFERENTIAL IMMUNOPHENOTYPE RECOVERIES AFTER IMMUNOMAGNETIC SEPARATION OF CD 34+ PROGENITOR CELLS FROM CORD BLOOD (CB) AND ADULT BONE MARROW (ABM). Carmella van de Ven, Ping Law, Adrian Gee, Yu Suen, Eva Knoppel and Mitchell S. Cairo, Baxter Healthcare Corp., Children's Hospital of Orange County, Orange, CA 92668

Bernstein et al (Blood 77:2316, 1991) has demonstrated the synergistic interaction between SCF and other CSFs on very immature populations of CD 34+ ABM progenitors. We compared the difference in the response of isolated CD 34+ progenitor cells from CB (neonatal) and ABM to SCF and other CSFs. CB was obtained during non-stressed normal vaginal deliveries or scheduled C-sections, and ABM during normal BM harvesting for BMT. Briefly, CD 34+ mononuclear cells were isolated following Ficoll-paque density gradient centrifugation, indirect immunomagnetic separation with HPCA-1 and Dynal paramagnetic microspheres coated with rat anti-mouse IgG1, and bead release with chymopapain. CD 34+ subsets were immunophenotyped on a Becton Dickinson FacStar with CD 34 (8g12), CD33 (Leu M9), CD 38 (Leu 17), HLA-Dr, CD 5 (Leu 1) and CD 19 (Leu 12). Isolated CD 34+ cells were plated (10^2/well) from both CB and ABM in 0.9% methylcellulose bone marrow kit (Gibco) supplemented with EPO (2U/ml) and stimulated with SCF, IL3, GM-CSF, or G-CSF (100 ng/ml). The percent CD34+ recovery was significantly higher in CB than ABM (99.35 ± 18% vs 26.3 ± 0.1%) ($p< 0.001$). CD 34+ populations in the CB and ABM were similar for CD33 (0.17 vs 0.63%), CD 38 (83.1 vs 91.7%), HLA-DR (92.2 vs 66.7%), and CD5 (1.17 vs 0.51%), however, there was a striking difference in B-cell lineage between CB and ABM CD 34+ cells (2.66 ± 0.8 vs 41.1 ±6.3 %) ($p<0.001$). Colony formation in the selected CD 34+ population was enhanced by the addition of SCF to IL3 with over a twofold increase in CFU-GM in CB and ABM (CB:10.3 ±1.5 *IL3* vs 26 ± 2.8 *IL3/SCF* ; ABM:4.3 ±1.5 *IL3* vs 11.7 ± 1.1 *IL3/SCF*) ($p<0.05$), and the addition of SCF to GM-CSF produced a fourfold increase in CFU-GM in CB (6.0 ± 3.0 *GM* vs 28.0± 1.7 *GM/SCF*) ($p<0.01$), versus a twofold increase in ABM (6.7±1.1 *GM* vs 16.3 ± 3.5 *GM/SCF*) ($p<0.01$). Furthermore, CB showed a threefold increase in CFU-GM when SCF was added to G-CSF (11.5 ±0.7 *G* vs 30.3 ± 6.8 *G/SCF*) ($p<0.05$), but no significant increase in CFU-GM was seen in ABM with G/SCF (10.7 ± 4.0 *G* vs 17.7 ± 9.0 *G/SCF*). These preliminary data suggest that following positive CD 34+ selection with immunomagnetic beads CB recovery is significantly higher, it is not associated with B lineage phenotype, and CB CD 34+ cells may be more sensitive to IL3/SCF, GM-CSF/SCF, and G-CSF/SCF stimulation than selected CD 34+ ABM.

M 340 THE HUMAN *KIT* GENE: STRUCTURAL FEATURES AND PROMOTER ANALYSIS, George R. Vandenbark, Carlos M. deCastro, Harold M. Johnson and Russel E. Kaufman, Department of Medicine, Duke University Medical Center, Durham, NC 27710. The finding that the c-*kit* gene and its ligand are allelic with the mouse *W* and *Sl* loci, respectively, illustrates their importance in embryogenesis, melanogenesis and hematopoiesis. In order to study the potential role of c-*kit* in human diseases, we have cloned the human *KIT* gene, characterized its structure and begun analysis of its promoter region. The gene is composed of 21 exons encoding 5230 bp of transcribed sequence which are contained within greater than 70 Kb of DNA. Except for the last, all exons are quite small. The introns are variable in size from greater than 30 Kb for intron 1 to 82 bp for intron 12. The overall structure and intron-exon boundaries are quite similar to those found in *FMS*. An alternate splicing pattern found by others is located at the 3' end of exon 9. Using pulsed field gel electrophoresis, we have linked the $PDGFR_A$ and KIT genes on a 700 Kb Bss HI fragment. A single transcription start site has been defined and which corresponds to the first base in the published cDNA sequence. The 5' flanking-promoter region contains neither a TATA element nor a CAAT element in the usual place. Preliminary functional studies of this promoter region have been performed using a chimeric luciferase reporter gene containing a series of 5' deletion promoter segments. By studying transient expression in c-*kit* expressing (HEL and MEG-01) and nonexpressing (KG-1) human hematopoietic cell lines, we found that tissue specific, positive cis-acting elements are contained within the promoter region from -37 to -182, relative to the transcription start site. Inclusion of DNA from about -500 to -5000 negatively influenced expression. The -37 to -182 area contains, among other potential elements, a consensus binding site for the GATA family of transcriptional activators. The expression of one member of this family, GATA-1, correlates with *KIT* expression in hematopoietic lineages and may be an important determinant in the transcriptional regulation of this gene.

M 341 C-MPL A NEW MEMBER OF THE HEMATOPOIETIC GROWTH FACTOR RECEPTOR SUPERFAMILY: CLONING AND EXPRESSION.
Isabelle Vigon, Serge Fichelson, Laurence Cocault, Sylvie Gisselbrecht and Michèle Souyri. ICGM, INSERM U152, Hopital Cochin, 27 rue du Fbg St Jacques, 75674 Paris Cedex, France.

The Myeloproliferative Leukemia Virus (MPLV) derived from the helper component of the Friend Murine Leukemia Virus (F-MuLV) has transduced a new oncogene named v-mpl. We have previously shown that this oncogene encodes a truncated form of a receptor-like molecule which belongs to the recently described hematopoietic growth factor receptor superfamily (Souyri et al, 1990, Cell 63:1137-1147). Human (h-mpl) and murine (m-mpl) cellular homologues of v-mpl have been cloned. From mice, we have isolated a complete cDNA clone which encodes a putative protein with an estimated molecular weight of 70 kD. The comparison between the amino acid sequences of v-mpl and its cellular counterpart revealed that they were 100% homologous. Several c-mpl cDNA species have been isolated from humans, probably due to alternative splicing, as previously described for most receptors of this family. We have cloned two cDNA species, clones P and K, coding for putative proteins whose estimated molecular weights are 71 and 65 kD, respectively. They share extracellular and transmembrane domains but differ in their cytoplasmic regions. c-DNA sequence analysis of human and mouse cDNA clones has shown that the extracellular domain contains the consensus sequences described for all members of this receptor superfamily. These clones will be presented and compared.

We have looked for mpl expression in murine tissues and this expression seemed present only in hematopoietic tissues. In order to determine the hematopoietic cells which preferentially express m-mpl, we have analyzed total RNAs from cell lines of various lineages, as well as from bone marrow and spleen cells previously enriched in hematopoietic precursors. Preliminary results suggesting that m-mpl could be preferentially expressed in a subpopulation of hematopoietic precursors, will be presented.

M 342 NEUTRALIZING ANTIBODY TO TGF-ß OVERCOMES NORMAL GROWTH CONTROLS IN LONG-TERM BONE MARROW CULTURES, Wendy O. Waegell, Howard R. Higley, Paul W. Kincade and James R. Dasch, Department of Immunology, Celtrix Laboratories, Palo Alto, CA 94303 Hematopoietic stem cell growth has been shown to be significantly inhibited by the addition of exogenous transforming growth factor beta to Dexter-type long-term bone marrow cultures. In order to examine whether TGF-ß produced by these cells has a role in hematopoietic growth regulation, Dexter cultures have been treated with a monoclonal antibody, 1D11.16, which neutralizes the biological activity of TGF-ß types 1, 2 and 3, or with a control antibody, MOPC 21C. The composition and cellularity of the nonadherent cell populations in these cultures were assessed weekly. Treatment with anti-TGF-ß Ab resulted in a five fold increase in nonadherent cells in the cultures as compared to either the MOPC control or untreated cultures. The majority of these cells were granulocyte/ macrophage precursors as assessed by histologic and FACS analysis. There was also a significant increase in megakaryocytes in cultures treated with anti-TGF-ß Ab. In addition, a CFU-S assay combining both the adherent and nonadherent populations from 6 week cultures showed that there are an equivalent number of hematopoietic stem cells per 10^6 cells regardless of antibody treatment. Finally, kinetics studies show that the presence of anti-TGF-ß Ab is required from the onset of culture to produce these effects. These results suggest that TGF-ß is involved in normal growth regulation of bone marrow hematopoietic cells. By addition of a neutralizing antibody, the normal TGF-ß negative growth signal is disrupted, allowing expanded growth of several cell populations.

M 343 EFFECTS OF RECOMBINANT TRANSFORMING GROWTH FACTOR β_1 ($rTGFB_1$) ON HEMATOLOGIC RECOVERY FOLLOWING TREATMENT OF MICE WITH 5-FLUOROURACIL. Robert H. Wiltrout, Rob Jansen, Giovanna Damia, Noriko Usui, Jonathan Keller, Dan L. Longo, and Francis W. Ruscetti, Laboratories of Experimental Immunology and Molecular Immunoregulation, Biological Response Modifiers Program, NCI-FCRDC, and Biological Carcinogenesis and Development Program, PRI/DynCorp, NCI-FCRDC, Frederick, MD 21702. Transforming growth factor β_1 ($TGF\beta_1$) has been shown to inhibit bone marrow (BM) colony formation following in vitro treatment as well as after in vivo administration to normal mice. These data suggest that $TGF\beta$ might either protect, or further depress, progenitor cell levels in mice exposed to a cell cycle-active drug such as 5-fluorouracil (5FU). The repeated intraperitoneal (ip) administration of recombinant $TGF\beta_1$ ($rTGF\beta_1$) during the hyperproliferative state of the bone marrow that occurs 7-9 days after the iv administration of 150 mg/kg 5FU inhibited the formation of both multilineage (CFU-GEMM) and the more differentiated (CFU-c) colonies by 20-40% per culture, and 66% to 93% per mouse. The administration of multiple doses of $rTGF\beta_1$ immediately prior to the injection of 5FU also inhibited the resulting rebound in the number of CFU-c and CFU-GEMM colonies per culture and the total number of CFU per mouse by 30-77%, and >90%, respectively. The formation of extra- medullary CFU-c and CFU-GEMM in the spleen was inhibited by >50% and >75%, respectively, by the repeated administration of $TGF\beta_1$. Further, the repeated administration of $TGF\beta_1$ for 5 days immediately preceding or following 5FU injection inhibited the number of high-proliferative potential colony-forming cell (HPP-CFC) progenitors by >75%. All of these effects of $TGF\beta_1$ on progenitor cells are dose-dependent with maximal inhibition of CFU occurring at a twice daily dose of ≥ 5 μg $TGF\beta_1$ administered for 5 days. Thus, these results demonstrate that CFU progenitor functions can be substantially inhibited in vivo by $rTGF\beta_1$ and suggest that these effects may be cumulative after chronic administration.

M 344 IL 11 EXPRESSION IN DONOR BONE MARROW CELLS IMPROVES HEMATOLOGICAL RECONSTITUTION IN LETHALLY IRRADIATED RECIPIENT MICE.

C. R. Wood, S. R. Paul[+], S. Goldman, M. Muench*, R. Palmer, G. Pedneault, G. E. Morris, M. Bree, L. Hayes, J. Hoysradt, K. McCarthy, T. Neben, F. Bennett, R. J. Kaufman, R. Schaub, M.A.S. Moore*, S. C. Clark,
Genetics Institute, Cambridge, MA., Dana-Farber Cancer Institute[+], Boston, MA. and Memorial Sloan-Kettering Cancer Center*, New York, NY.

Interleukin 11 (IL 11) is a newly described stromal cell-derived hematopoietic growth factor, with effects on in vitro megakaryocytopoiesis and lymphopoiesis. In order to evaluate the in vivo effects of this growth factor, we have constructed a recombinant retrovirus containing a neo gene and human IL 11 cDNA. Bone marrow cells derived from mice pre-treated with 5-fluorouracil have been infected with this retrovirus and then transplanted into lethally irradiated recipient mice. The reconstitution of these mice was then examined relative to control mice transplanted with bone marrow cells either uninfected or infected with a retrovirus expressing only neo.

Platelet reconstitution was found to be more rapid in mice rescued with IL 11 retrovirus infected-bone marrow than in control mice. At 14 days after transplantation, the recipients of IL 11 retrovirus infected-bone marrow had over two-fold higher levels of circulating platelets. Preliminary studies have indicated that this increase correlates with an increased number of CFU-Meg in bone marrow and spleen. Marrow from transplanted mice, following 7 day suspension culture with IL 1β and kit ligand, generated secondary high proliferative potential-colony forming cells (HPP-CFC). IL 11 retrovirus infected marrow had an up to 8-fold increased capacity to generate these secondary HPP-CFC relative to control marrow.

M 345 THE FUNCTION OF THE FES TYROSINE KINASE IN HUMAN MYELOID CELLS, Karen E. Yates, Maureen R. Lynch and Judith C. Gasson, Departments of Biological Chemistry and Medicine (Hematology-Oncology), UCLA School of Medicine, Los Angeles CA

Fes is a member of the *src* family of non-receptor cytoplasmic tyrosine kinases. Although it contains the tyrosine kinase and SH2 domains, this protein lacks some of the structural features common to other *src*-like kinases; thus, it is thought that Fes diverged earlier than the rest of the family, and may function in a somewhat different manner. c-*fes* mRNA has been observed in both normal and leukemic cells of the myeloid lineage, with the greatest expression seen in monocytes and neutrophils. Anti-sense RNA experiments have suggested that Fes may be involved in proliferation and/or maturation of myeloid cells, and we are interested in the function of Fes in these processes. Currently, we are using cell fractionation techniques to determine the subcellular localization of Fes protein in primary human neutrophils and the factor-dependent cell line, TF-1. Preliminary studies show that Fes associates with the plasma membrane and nuclear fractions; no Fes protein is detected in S45 cytosol. These results are in contrast to the reported cellular distribution for *src*, which is found primarily in the plasma membrane and cytosol. Additional ongoing studies aim to elucidate the role of Fes in myeloid cell growth and maturation.

Cytokines/Microenvironment; Red Cells

M 400 CLONING OF HUMAN cDNA ENCODING FOR A REGULATORY PROTEIN (GDI) OF THE rho PROTEINS, ras-LIKE G-PROTEINS. Chaker N. Adra, Jean-Michel Lelias, Jean-Claude Guillemot, Gerburg M. Wulf, Bing Lim. Beth Israel Hospital/ Harvard Medical School, Boston, Massachusetts.

During the differential screening of human hematopoietic cDNA libraries enriched for "hematopoieitc specific" sequences by subtractive hybridization, we isolated a clone designated D4, which detects an mRNA expressed at high level in all human cells representing the various hematopoietic lineages. A 1.9kb transcript was detected in the Northern blot analysis of total RNA from cell lines of Erythroid (OCIR, HEL), Granulocytic (HL-60, KG1), T-Lymphoid (Molt4), B lymphoid (My-1), Mixed-lineage (K562) origin and from normal human bone marrow. In a similar analysis of cell lines of non-hematopoietic origin, the transcript was either expressed at much lower levels (bone-marrow stroma, lung, melanoma) or non-detectable (liver, cervical, skeletal muscle and neuronal). The full length cDNA encodes for a molecule with 66% identity to the recently described bovine rho-GDI [GDP-dissociation inhibitor] protein and cDNA. Clone D4 also detects cross-hybridizing transcripts in murine mRNA. The rho protein family belongs to an evolutionarily well-conserved superfamily of ras p21/ras p21-like small GTP-binding proteins (G proteins). While the physiological functions of rho protein have not been defined, there is evidence for their participation in the regulation of cell growth, morphology and tumorigenesis. Biochemically, rho appears to activate the GTP/GDP complex by binding to GTP. Rho-GDI inhibits the dissociation of GDP from the GTP/GDP complex and thereby, prevents the formation of the active rho-GTP complex. To begin examining the significance of the overexpression of rho-GDI in hematopoietic cells, we studied its expression during cellular differentiation using an in-vitro assay developed to examine the differentiation of murine totipotent embryonal stem (ES) cells into hematopoietic cells. We found that rho-GD1 appears to be upregulated during the transition of ES cells into hematopoietic cells. However, in hematopoieitc cell lines induced to undergo further differentiation with chemical inducers, rho-GD1 is down-regulated with differentiation. To further elucidate the significance of these observations, we are currently studying the effect of dysregulating rho-GDI expression on the development and proliferation of hematopoietic cells.

M 401 OVER-EXPRESSION OF C-SRC OR V-SRC IN MARROW STROMAL CELLS STIMULATES PROLIFERATION OF PROGENITORS IN LONG TERM MARROW CULTURE. Steven M. Anderson and Jeanette Mladenovic, Depts of Pathology and Medicine, SUNY Stony Brook, NY 11794 and VAMC, Northport, NY

The murine bone marrow stromal cell line S17 was infected with retroviruses expressing either *v-src* or *c-src* and long term marrow cultures (LTBMC) were established with these stromal cells to delineate the effect of *src* upon proliferation of progenitor cells. A three- to five-fold increase in the number of nonadherent cells, granulocytes and monocytes was observed in LTBMCs prepared with either S17/v-src or S17/c-src stromal cells compared to LTBMC with normal S17 cells. A corresponding increase in the number of CFU-GM was also observed in LTBMCs prepared with either S17/c-src or S17/v-src cells. In all cases, the increase in the number of cells was 2-3 fold greater in LTBMC established with S17/c-src stromal cells than with S17/v-src cells. Conditioned media (CM) from S17/c-src or S17/v-src cells stimulated formation of CFU-GM and enhanced formation of both BFU-E and CFU-GEMM by bone marrow cells. CM from S17, S17/v-src and S17/c-src cells were tested for their ability to stimulate proliferation of factor-dependent cell lines (32D cl3, FDCP-1 and NFS-60). A dose-dependent stimulation of FDCP-1 cells was observed with CM from either S17/v-src or S17/c-src cells. No stimulation of 32D cl3 or NFS-60 cells was observed. This growth stimulatory activity was completely neutralized by the addition of 1μg/ml anti-GM-CSF monoclonal antibody, but not anti-IL-3 or anti-IL-4 monoclonal antibodies. Titration assays indicate that S17 cells produce ≤0.0025 ng/ml GM-CSF; S17/v-src, 0.025 ng/ml; and S17/c-src, 0.125 ng/ml GM-CSF. By PCR analysis all three cell lines contain mRNA for GM-CSF, IL-6 and stem cell growth factor (ligand for *c-kit*). No IL-3 mRNA was detected. These data suggest that *src*-stimulated production of GM-CSF may be responsible for the increased proliferation of progenitors. The ability of *c-src* to stimulate production of GM-CSF was surprising since it is a nonactivated proto-oncogene. Our results also suggests that oncogene-induced paracrine growth cycles involving bone marrow stromal cells may contribute to leukemogenesis.

M 402 BONE MARROW RESPONSE TO A TRYPANOSOMA CONGOLENSE RECHALLENGE INFECTION IN BORAN CATTLE, Aurélie A. Andrianarivo, Linda L. Logan-Henfrey, Paul Muiya and Francis A. McOdimba, International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya.

The pathogenesis of the anaemia associated with bovine trypanosomiasis is still obscure. Using clonal assays for the committed hemopoietic progenitors, we have examined the bone marrow response to a Trypanosoma congolense rechallenge infection in trypanosusceptible Boran cattle. Very early in the infection (weeks 2 and 3) in the absence of any detectable parasitemia, the number of nucleated marrow cells dropped significantly. A concomittant marked decrease in both the early (BFU-E) and late (CFU-E) erythroid progenitors, as well as the granulocyte-macrophage precursors (CFU-GM) suggests a defect at the pluripotential cell level. Subsequently, while the CFU-E started to recover, the BFU-E remained low throughout the infection. Despite the CFU-E recovery, the packed cell volume (PCV) levels progressively declined and three out of the five animals studied developed chronic anaemia. No erythroid progenitors have been detected in the peripheral blood. These data seem to reflect different control systems for the two erythroid progenitors later on in the infection and appear to agree with previous results obtained in our laboratory. We have shown that medium conditioned by lectin (concanavalin A or phytohaemagglutinin)-stimulated peripheral blood leucocytes selectively inhibited the growth of bovine marrow BFU-E with little or no effect on CFU-E. We and others (Kaaya et al., 1982) have also demonstrated that bovine marrow CFU-E were very sensitive to erythropoietin. The trace level present in the fetal calf serum used in the clonal assays was sufficient to induce between 20 to 50% of the CFU-E scored in the presence of exogenous erythropoietin.

The mechanisms and factors responsible for the altered bone marrow function as described in this study remain to be established. However these findings are useful and will allow future comparative studies of bone marrow responsiveness in trypanosusceptible and trypanotolerant cattle.

Kaaya G.P., Jamal N., Maxie M.G. and Messner H.A., Research in Veterinary Science, 32, 213-220 (1982).

M 403 EXPRESSION OF SELECTINS ON HUMAN CD34+ PROGENITOR CELLS AND THEIR INVOLVEMENT IN MEDIATING ADHESION OF STEM CELLS TO MARROW STROMA, Anklesaria P., Pratt D., Department of Radiation Oncology, University Massachusetts Medical Center, Worcester, MA 01655.

Human stem cells expressing CD34 home and seed into bone-marrow microenvironment. We have established primary human stromal cell cultures that support long term adhesion, proliferation and differentiation of cocultivated $CD34^{hi}$ progenitor cells. These stromal cell cultures express IL-1, but not IL-3, IL-6, IL-4 or IL-5. Using a positive selection process $CD34^{hi}CD33^{lo}$ cells were enriched from normal bone marrow. These enriched cells form blast cell colonies at high plating efficiencies (20-30%) *in vitro* in response to MGF, IL-3 and EPO and can also initiate long -term cultures. These data indicate that enriched $CD34^{hi}$ population contain a sub-population of primitive stem cells. We have also demonstrated that these enriched $CD34^{hi}$ cells utilize ß1 and ß2 integrins and their ligands to adhere to human marrow stromal cells. LAM-1 a member of the selectin family of adhesion molecules was expressed on normal marrow $CD34^{hi}$ progenitors. In contrast the committed $CD34^{lo}CD38^{hi}$-$CD33^{hi}$ progenitors expressed low levels of LAM-1. A $CD34^{+}$ human factor-dependent cell line KMT2 also expressed LAM1 while the receptor for ELAM-1 was undetected. These data indicate that $CD34^{+}$ cells differentially express receptors of the selectin family and may utilize them to home and adhere to marrow stromal cells. Expression of these receptors are now being evaluated on leukemic cells.

M 404 STEM CELL FACTOR (SCF) MARKEDLY INCREASES ERYTHROID COLONY FORMATION IN MDS, Bianca Backx, Lianne Broeders and Bob Löwenberg, The Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

The myelodysplastic syndromes (MDS) represent a heterogenous group of hematological disorders characterized by abnormal maturation involving one or more of the marrow lineages. In MDS in vitro colony formation of bone marrow progenitors is markedly reduced. Our interest was to study the effect of the recently cloned stem cell factor (SCF) alone or in combination with other hematopoietic growth factors on highly enriched MDS marrow progenitors. Bone marrow cells depleted of myelo-monocytic and T-cells, were positively selected (CD34) by cell sorting and cultured for 14 days in a methyl cellulose system. In only 1 out of 9 MDS cases (RAEB) SCF alone induced colony formation (erythroid). In 6 of 9 cases (2 RAEB, 2 RAEB-t and 2 CMML) the addition of SCF to erythropoietin (EPO) plus interleukin-3 (IL-3) cultures resulted in a 2 to 3 fold increase in erythroid colony formation as compared to colony numbers induced by Epo plus IL-3. In 3 cases (2 RA, 1 RARS) the addition of SCF resulted in a more than 10 fold increase. The effect of SCF on granulocytic colony-stimulating factor (G-CSF) induced colony formation appeared to be comparable in all MDS cases tested, namely a 2 to 3 fold increase in granulocytic colony formation. These results suggest that SCF can be an important factor in restoring the erythroid respons in at least a subgroup of MDS patients.

M 405 HIGH LEVEL OF EXPRESSION OF HUMAN GLUCOCEREBROSIDASE IN MACROPHAGES CULTURED FROM RETROVIRALLY TRANSDUCED STEM CELLS, Sallie S. Boggs, Paul D. Robbins, Alfred B. Bahnson, Kenneth D. Patrene, Huiling He, Toya Ohashi, Mark Kimak, Shelly Clark, and John A. Barranger, Departments of Radiation Oncology, Human Genetics, and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine and the Pittsburgh Cancer Institute, Pittsburgh, PA 15261

Gaucher disease is caused by abnormal lysosomal storage of glucosylceramide in macrophage cells due to a deficiency of glucocerebrosidase (GC). Although transfer of the GC gene to murine stem cells has been accomplished previously, expression of the gene product has been poor, and no expression has been demonstrated in the target macrophage cells. Using a MFG-GC retrovirus vector, high levels of GC expression were seen in 100% of the $CFU\text{-}S_{12}$. Infected bone marrow seeded onto irradiated hematopoietic stroma produced neutrophils and monocytic macrophage cells that expressed high levels of GC enzyme. Bone marrow from mice surviving at least 5 months with > 90% donor MFG-GC-infected hematopoietic cells, cultured in the presence of L cell conditioned medium, produced functional macrophages with high GC activity. Thus, we have demonstrated, for the first time, high levels of human GC expression in macrophages derived from long-term marrow repopulating stem cells.

M 406 CULTURE OF HUMAN HEMOPOIETIC PROGENITOR CELLS ON CLONED STROMAL CELLS TRANSFORMED BY SV40 LARGE T ANTIGEN UNDER THE CONTROL OF AN INDUCIBLE PROMOTER

Andrew W.Boyd, Flavia M.Cicuttini, C.Glen Begley, Leonie Ashman & Michael Martin. Lions Cancer Research Lab., Walter & Eliza Institute, Royal Melbourne Hospital, 3050, Australia.

Human stromal cells were immortalized by the SV40 large T antigen under the control of a synthetic metallothionein promoter. The clones were isolated in the presence of 100µM $ZnCl_2$ and subsequently maintained and expanded under these conditions. All clones returned to contact inhibitable growth after removal of Zn and could be maintained as stable, functional monolayers for many months. When pure populations of CD34-positive cells were seeded onto these stromal layers, proliferation of the non-adherent hemopoietic cells was observed, and cells capable of colony formation in secondary agar cultures could be recovered for several months. Several lines showed greater capacity to support CFC than normal, mixed stromal layers. These lines were characterized by very low production of G- and GM-CSF. Moreover, when GM-CSF was added to these co-cultures, while there was an initial enhancement of proliferation, there was more rapid decline in colony numbers. The colony types recovered altered with time, the early time points yielding cells capable of forming mixed cell colonies as well as GM-, G- and M-CFU. At later time points the mixed colonies disappeared and the size of the granulocyte-macrophage colonies was reduced.

We have used this relatively simple culture system to probe a number of potential regulatory molecules. in particular, when antibodies to c-kit were added to the cultures, complete inhibition of hemopoiesis was seen, while parallel cultures to which isotype-identical antibodies were added showed no difference from control cultures. All the lines produced SCF(c-kit ligand) mRNA, but no SCF was detectable in supernatants, leading us to the conclusion that c-kit expressed on progenitor cells was interacting with either membrane or matrix bound SCF on the stromal lines.

M 407 THE PRODUCT OF THE *vav* PROTO-ONCOGENE DEFINES A NOVEL CLASS OF TYROSINE PROTEIN KINASE SUBSTRATES IN HEMATOPOIETIC CELLS.

Xosé R. Bustelo and Mariano Barbacid, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

The *vav* proto-oncogene was first identified in our laboratory by virtue of its ability to transform NIH3T3 cells in gene transfer assays (EMBO J. 8, 2283, 1989). Subsequent studies demonstrated that the *vav* gene encodes a 95 kDa protein,$p95^{vav}$, which is specifically expressed in hematopoietic cells regardless of their differentiation lineage. Here we report that $p95^{vav}$ contains a SH2 domain and is a substrate for tyrosine protein kinases. Ectopic expression of $p95^{vav}$ in NIH3T3 cells leads to constitutive phosphorylation of this protein on tyrosine residues in actively growing cells. Exposure of quiescent NIH3T3 expressing $p95^{vav}$ to either EGF or PDGF induces the rapid phosphorylation of this protein on tyrosine residues. Moreover, activation of the EGF and PDGF receptors by their cognate ligand results in their physical association with $p95^{vav}$, a process which is mediated exclusively by its SH2 sequences. In T-cells, co-activation of the T-cell receptor and the accesory CD4 cell surface protein also results in the phosphorylation of the endogenous $p95^{vav}$ protein on tyrosine residues. Similar to the situation in T-cells, signal transduction mediated thorugh the B-cell antigen receptor also involves tyrosine phosphorylation of $p95^{vav}$. These results indicate that this proto-oncogene plays an imporant role in the activation process of lymphoid cells. In addition to the SH2 domain, $p95^{vav}$ contains an array of structural motifs so far not found in other tyrosine kinase substrates. Those motifs include an HLH/leucine zipper-like domain which exhibits limited sequence homology with those present in the Myc and Max proteins, an acidic domain, and a cystein rich region with homology to the phorbol ester binding domains of protein kinase C and n-chimaerin. These observations indicate that $p95^{vav}$ represents a new class of signal transduction molecules and suggest a possible role for this protein in the transduction of tyrosine phosphorylation signalling into transcriptional events in hematopoietic cells.

M 408 REGULATION OF BONE MARROW CELL CYCLE STATUS, CELLULARITY, AND MYELOID PHENOTYPE BY SUBCUTANEOUS AND INTRAPERITONEAL ADMINISTRATION OF rhTGF-ß2.

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In vitro colony formation by hematopoietic progenitors is regulated in a bidirectional fashion by Transforming Growth Factor-Beta (TGF-ß). We have begun in vivo evaluations of TGF-ß2 suppression of bone marrow and splenic white blood cells in order to assess its use for protection of early and mid-progenitors from chemotherapeutic drug toxicity. C3H mice were treated by subcutaneous (SQ) or intraperitoneal (IP) injections of rhTGF-ß2 over 1-14 days, and were evaluated for: bone marrow and splenic cell cycle status and phenotype by FACS, organ cellularity, and body weight. One to three daily IP injections of 1-100µg of TGF-ß2 resulted in a significant increase in the percentage of cells in G_0/G_1, as well as a decrease in cells in both S and G_2/M to ≤3% of all cells. The response to a single IP injection of TGF-ß2 was maximal after ~24 hours. The SQ route was effective only when the cytokine was administered for extended periods of time. Decreases in immune organ cellularity and body weight occurred in a route- and dose-dependent fashion, with no decrease in cell viability. Two-color FACS analysis using the DNA stain propidium iodide and the anti-granulocyte antibody 8C5 revealed that cells of the granulocyte lineage are growth inhibited such that there is a greater percentage of more mature, $8C5^{hi}$ cells in G_0/G_1 following TGF-ß2 treatment. These results indicate that the utility of TGF-ß2 as a bone marrow suppressant and chemoprotectant will most likely be dependent upon the dose, schedule, and route of administration selected.

M 409 EVIDENCE FOR AN INDIRECT MECHANISM MEDIATING THE INHIBITORY EFFECT OF THE TETRAPEPTIDE AcSDKP ON PRIMITIVE HUMAN HEMATOPOIETIC CELL PROLIFERATION.

Cashman, J.D., Eaves A.C., and Eaves C.J. Terry Fox Laboratory, B.C. Cancer Agency and University of B.C., Vancouver, B.C. Canada.

The long-term culture (LTC) system permits the maintenance and turnover of primitive hematopoietic cells for several weeks in the presence of a stromal cell-containing adherent layer and has proven useful for analyzing stromal cell-mediated mechanisms controlling primitive hematopoietic cell proliferation. In previous experiments we have shown that the ability of unperturbed stromal cells to prevent adjacent primitive hematopoietic cells from entering S-phase can be overcome either by the addition of fresh horse serum or a number of mesenchymal cell activators, or by the addition of an antibody capable of neutralizing TGF-β, an endogenously produced inhibitor whose effects can be shown to mimic those of unperturbed stromal cells. Recently, we have tested the ability of a putative inhibitory tetrapeptide, AcSDKP, to alter the cycling activity of primitive human hematopoietic cells in the LTC system. Addition of 100-300ng/ml of AcSDKP to established cultures at the time of the regular weekly half-medium change was sufficient to reversibly and selectively block the entry into S-phase of high proliferative potential erythroid and granulocyte progenitors in the adherent layer which occurred in control cultures to which only fresh medium was added. Addition of fresh medium plus a control tetrapeptide (AcSDKE), or the unacetylated form of the active peptide (SDKP), allowed the same stimulation of proliferation seen in control cultures. The effect of these peptides on hematopoietic colony formation by normal human marrow buffy coat cells in methylcellulose assays containing each tetrapeptide was also examined. At concentrations ≥100ng/ml a significant inhibitory effect of AcSDKP (but not of AcSDKE) was observed on erythroid progenitors and on high proliferative potential CFU-GM. In parallel methylcellulose assays, a decrease in colony formation was also obtained by the unacetylated form of the active tetrapeptide suggesting that the acetyl group was not absolutely necessary for the inhibition seen under these conditions. Methylcellulose assays of marrow cells from which adherent cells had been selectively removed failed to show an inhibitory effect of AcSDKP suggesting that this molecule acts indirectly by causing the production or release of a direct-acting inhibitor(s) from adherent marrow cells.

M 410 HYBRID TRANSCRIPT JUNCTIONS IN APL: CORRELATION WITH GENOMIC BREAKPOINTS,

David F. Claxton, Lalitha Nagarajan, Y. Tsujimoto, Chris Reading, Elihu Estey, Borje Andersson, Albert B. Deisseroth, Department of Hematology, MD Anderson Cancer Center, Houston, TX 77030 and Wistar Inst, Philadelphia, PA 19104

Acute promyelocytic leukemia (APL), the FAB M3 form of acute myelogenous leukemia, is nearly always associated with the balanced chromosomal translocation t(15;17)(q22;21). This mutation has been shown to fuse PML, a novel chromosome 15 encoded gene, with the previously described retinoic acid receptor alpha (RARα) located at 17q21. PML-RARα fusion transcripts and gene products are expressed in leukemic cells. The fusion transcripts encode variable amounts of PML sequence upstream of functional RARα DNA and ligand binding domains. We have studied a series of cases of APL with "reverse" PCR using PML upstream primers and RARα downstream primers. Of 12 cases in which RNA was studied, 6 were found to have 3' PML splice junctions and 6 showed 5' junctions. Normal PML sequences are also amplifiable from these samples and, given their expression in normal and leukemic bone marrow, provide a useful positive control for cDNA quality. These results were correlated with genomic mapping of chromosome 15 translocation breakpoints using the genomic probe K3. Rearrangement of the 12Kbp BamHI or the 6Kbp HindIII hybridizing fragments was predictive of a 5'junction. In contrast, rearrangement downstream of the HindIII site was predictive for the 3' junction. Thus, we have shown that APL express one of two forms of PML-RARα hybrid transcript in the majority of cases. The type of transcript may be predicted on the basis of rearrangements seen with genomic Southern blots. Further study and longer followup of patients will be required to establish the biological and clinical relevance of these observations.

M 411 CELL CULTURE MEDIUM: EFFECTS ON HUMAN BONE MARROW PROGENITOR CELLS.

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The optimal conditions to collect, hold, and/or transport harvested human bone marrow (BM) for short periods of time (days) remain unresolved. This study examines culture medium effects on freshly harvested human BM, and investigates various marrow holding conditions and their effects on viability and CFU potential of known progenitor cell populations. BM aspirates from normal donors were harvested and either whole marrow or selective flow cytometry-sorted populations were placed immediately in either PBS, RPMI-1640 or Medium 199 (GIBCO, Grand Island, NY) supplemented with 20U/ml of preservative free heparin at a ratio of 4 parts BM to 1 part holding medium. Evaluations consisted of trypan blue staining for viability, CFU assays and immunophenotyping by flow cytometry analysis. BM samples were incubated under sterile conditions at room temperature or 37°C for 0.25, 1, 2, 3, 6, 12, and 18 days. Following each period of incubation, cell viability was ascertained, and Ficoll-Hypaque isolated mononuclear cells were immunophenotyped and plated in CFU assays. Preliminary results suggest that human BM can be held at room temperature for at least 3 days and still demonstrate the capacity to form both granulocyte-monocyte and erythroid colonies in numbers equal to those seen at the time of the initial culture. Alterations in $CD34^+$ cells and other cells appear to be donor and condition dependent. Currently, we are analyzing results from CFU assays with those obtained from multiparameter flow cytometric studies. These data promise to provide useful information with regard to suitable conditions for maintaining human BM following harvest.

M 412 CYTOKINES AND EARLY HEMATOPOIETIC DEVELOPMENT,

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Two different approaches have been used to study the role of cytokines in the early development of hematopoietic cells during embryogenesis. Mouse blastocysts, after approximately eight days in culture, will form visible blood islands containing mature erythroid cells. We have used the genes for hematopoietic growth factor receptors and specific transcription factors as markers for committment and differentiation to hematopoietic cell lineages. The expression of these mRNAs has been assessed both in blastocysts and embryos cultured for various periods *in vitro*. In addition, blocking antibodies to certain cytokines were added to assess their effect on *in vitro* development, blood island formation, and expression of hematopoietic markers. The second approach involves the disruption of cytokine genes through homologous recombination in mouse embryonic stem cells. Currently, we are focusing on cytokines known to act on very early hematopoietic cells.

M 413 THE EFFCTS OF CYTOKINES ON BONE MARROW CD34+ AND STROMAL CELLS,

Ruigao Fei, Shelly Heimfeld, Ronald J Berenson, CellPro, Inc., Department of Immunobiology, Bothell, WA 98021

The proliferation and differentiation of hematopoietic cells can be regulated by a number of different cytokines. We have used a suspension culture system to analyze the effects of certain recombinant growth factors on enriched human bone marrow CD34+ cells isolated using the CEPRATE™ SC clinical cell separator. The results suggest that optimal expansion of progenitor cells occurs using a combination of stem cell factor(SCF), IL-3 and GM-CSF. This expansion does not occur if unseparated marrow cells are grown under the same conditions. After three weeks in suspension culture, the total number of CFU-GM increased 10-12 fold over the starting value from the isolated CD34+ cells. In the same period, the unseparated marrow cells had lost all CFU-GM activity. Analyzing the CD34+ cultures at earlier timepoints, CFU-GM increase 10-15 fold by day 7 and 40-50 fold by day 14. HPP-CFC numbers are increased 40-50 fold by day 7.

Bone marrow stromal cells contribute to the hematopoietic microenvironment *in vivo*. Some cytokines can be produced by these stromal cells, and the stromal cells themselves can proliferate in response to these factors. We have examined the proliferation of murine bone marrow endothelial-like cells in the presence of such factors. The results indicate that, similar to the hematopoietic progenitor cells, combinations of factors give a larger response. The combination of IL-1B, IL-3, and SCF showed the greatest proliferation, which may relate to the known competency inducing effect of SCF.

M 414 ACTIVITY OF RECOMBINANT HUMAN STEM CELL FACTOR (rHU-SCF) ON IN VITRO PROLIFERATION OF ACUTE MYELOID LEUKEMIA (AML) CELLS,

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rHu-SCF, a cytokine described to act on early hematopoietic progenitors (Martin et al., Cell 63: 203, 1990), was tested on highly enriched human AML cells. rHu-SCF (10-100 ng/ml) stimulated H^3-Tdr incorporation in liquid culture in 13/14 AML cases (median stimulation index 4.0). Its activity was comparable to that of GM-CSF, G-CSF and IL3, which could be potentiated in most cases by rHu-SCF. Colony growth in semisolid medium was strongly stimulated by rHu-SCF alone in 3 case, one almost unresponsive to the other cytokines. In the other 11, rHu-SCF was quite less effective than GM-CSF and IL-3; however in 50-60% of cases it increase GM-CSF, IL3 and G-CSF induced colony growth. CFU-L maintenance was studied by culturing AML cells for 7 days, in liquid medium with and without growth factors. 25µl of cell suspension were then plated in semisolid medium with 5637 CM for colony assay. In all cases rHU-SCF enhanced CFU-L recovery by the median factor of 2.7, significantly higher than that achieved with GM-CSF, G-CSF, IL-3. A further increased CFU-L recovery was generally observed in the presence of a combination of those factors with SCF. rHu-SCF is therefore higly effective in stimulating AML cell growth and CFU-L self mainteining. It could play a role in the in vivo growth of AML cells.

M 415 TRANSFERRIN RECEPTOR mRNA REGULATION DURING ERYTHROID DIFFERENTIATION,

Roxanne Y.Y. Chan, Herbert M. Schulman and Prem Ponka, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2

In proliferating non-erythroid cells the expression of transferrin receptors (TfR) is regulated in an inverse manner by the amount of intracellular iron. Fe-dependent regulation of TfRs occurs post-transcriptionally and is mediated by iron responsive elements (IREs) located in the 3' UTR of the TfR mRNA. IREs are recognized by a specific cytoplasmic binding protein (IRE-BP) that in the absence of Fe binds, with high affinity, to TfR mRNA preventing its degradation. While TfR numbers are positively correlated with proliferation in non-erythroid cells, in hemoglobin-synthesizing cells their numbers increase during differentiation and are, therefore, negatively correlated with proliferation. This suggests a distinct regulation of erythroid TfR expression and further evidence for this claim was found in the present study: 1) With nuclear run on assays our experiments show increased TfR mRNA transcription following murine erythroleukemia (MEL) cell treatment with DMSO. If receptor induction is a consequence of cellular iron depletion due to Fe consumption for heme synthesis, then TfR transcription rates would not increase. 2) DMSO treatment of MEL cells does not increase IRE-BP activity which is, however, increased in uninduced MEL cells by Fe chelators. 3) Following induction of MEL cells there is an increase in the stability of TfR mRNA whose level is only slightly affected by iron excess. 4) Heme synthesis inhibitors, such as succinylacetone (SA), which inhibit numerous aspects of erythroid differentiation also inhibit TfR mRNA expression in induced MEL cells. The possibility that receptor expression is inhibited by nonheme Fe accumulated as a consequence of SA-inhibited heme synthesis was ruled out by our results showing that the cellular ferritin content does not change. However, SA does not decrease TfR mRNA levels in uninduced MEL cells. These studies thus indicate that TfR gene expression is regulated differently in hemoglobin synthesizing as compared to uninduced MEL cells.

M 416 A MUTATIONAL ANALYSIS OF THE EPO RECEPTOR

D.J. Hilton, S.S. Watowich, L. Katz and H.F.Lodish.

Erythropoietin (Epo) is a polypeptide hormone that plays a central role in the regulation of red blood cell formation. Epo elicits its effect through interaction with a receptor expressed on the surface of responsive cells. A cDNA molecule encoding the murine epo receptor was cloned in this laboratory in 1989 and is a member of a newly defined receptor family, that includes the receptors for other cytokines, such as interleukins 2-7, granulocyte and granulocyte-macrophage colony-stimulating factors and growth hormone. While these receptors do not exhibit homology to G-protein coupled receptors or receptor tyrosine kinases they do share certain structural motifs - most notably four cysteine residues and the sequence WSXWS in the extracellular region.

The structural motifs within the epo receptor required for efficient biosynthesis, correct folding, binding of epo and transmission of a biological are ill-defined. In order to gain further understanding of these critical regions deletion mutants have been created in which various portions of the extracellular and cytoplasmic domains have been removed. The function of the conserved WSXWS motif is also being examined by replacing the codons encoding the five residues with the sequence NN(GC), to yield mutant receptors with all possible 20 naturally occurring amino acids. Analysis of both classes of mutants is ongoing and includes the characteristics of their biosynthesis and ability to bind epo upon transient expression in COS fibroblasts and their capacity to transduce a biological signal when stably introduced into the factor-dependent cell line Ba/F3. In the latter experiments, the epo system offers distinct advantages over other cytokine receptors because activation can be achieved either by the addition of epo itself, the introduction of an activating mutation (R-->C) at position 129 or by transfection of the cells with the envelope protein of the spleen focus-forming virus.

M 417 HOX 2 HOMEOBOX GENES INFLUENCE RED CELL PHENOTYPE.

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We previously reported: HOX 2.2 homeobox mRNA decreases while HOX 2.6 increases during erythroid differentiation of leukemic cells in vitro; and over-expression of the HOX 2.2 gene in K562 or HEL cells causes a diminution of the erythroid phenotype. We now show that: 1) transfection of K562 cells with a vector over-expressing the HOX 2.6 homeobox cDNA produces an increase in several erythroid cell markers - heme content in liquid culture and benzidine staining of colonies grown in semi-solid media, and glycophorin A surface marker; also HOX 2.6 over-expressing cells form adherant cells compared to vector transfected controls. 2) transfection of K562 cells with a series of HOX 2.2 deletion mutants or mutants containing switched HOX 2.2 and HOX 2.1 homeobox domains show more complex phenotypic changes. Mutant cDNAs lacking either the homeobox or the extended N-terminal flanking domain are incapable of replicating the erythroid diminishing effects of the intact HOX 2.2 cDNA, while the N-terminal flanking region alone is able to compete effectively with the full-length HOX 2.2 protein. In addition, replacement of the HOX 2.2 box with HOX 2.1, ablates the observed phenotypic change, suggesting a specific HOX 2.2 binding site directed effect on red cell phenotype. 3) antisense HOX 2.1, 2.2, or 2.3 oligonucleotides specifically decrease erythroid colony formation, but not CFU-GM colonies from normal bone marrow or cord blood precursors.

M 418 DNA CLONING OF HUMAN HEMATOPOIETIC SPECIFIC (HS) GENES. Bing Lim, Jean-Michel Lelias, Chaker N. Adra, Jean-Claude Guillemot, Gerburg M. Wulf. Beth Israel Hospital/Harvard Medical School, Boston, Massachusetts.
Genes specifically expressed in particular cell types have been shown to govern critical developmental steps and biological functions unique to those cells. The hematopoietic system presents a plethora of diverse lineages each with a unique composite of transcribed genes that regulate their unique function. However, there is evidence that the differentiated lineages continue to remain related to each other after divergence from the hematopoietic stem cell. Genes specifically expressed and common to all hematopoietic cells would represent the molecular basis of such a link between lineages. The identification of such genes will provide a means of focusing on the regulatory molecules that may be critical for the development of the hematopoietic system as a whole. We have isolated several cDNA clones unique to hematopoietic cells by the differential screening of cDNA libraries of human hematopoietic cells (enriched for HS sequences by subtractive hybridization) with probes similarly enriched for HS sequences. Using cDNAs from these clones as probes, we screened Northern blots containing total RNAs from hematopoietic cell lines of lymphoid and myeloid origin and non-hematopoietic cell lines representative of epithelial, neuroectodermal, ectodermal and mesenchymal origin. Several clones were identified which detect mRNA transcripts of different sizes, in hematopoietic cells only. From the nucleotide information obtained by partial sequencing, we ascertained that some of the clones are the recently identified hematopoietic specific genes such as GATA-1 and HS-1. The others are not found in the GenBank database. Clone 13.5 detects a 2kb transcript in Myeloid cells (Erythroid and Granulocytic) which was not present in Lymphoid cells. This mRNA was down regulated when U937 (monocytic line) cells were induced to undergo differentiation into macrophages using phorbol ester (TPA). Clone 1.6 detects a 2.5kb long message that was present universally in hematopoietic cell lines of lymphoid (T and B) and Myeloid origin. In contrast to the 13.5-transcript, there was little change in the expression of 1.6 during differentiation of induced U937 cells. DS4 is another clone which detects a 4.5 transcript in both lymphoid and myeloid cells. These novel HS genes will be useful in investigating the molecular determinants of functions and development unique to hematopoietic cells. We are currently obtaining full length sequencing information for further structural and functional analysis.

M 419 WHAT IS THE EFFECT ON HEMATOPOIESIS OF A CONSTITUTIVELY ACTIVATED ERYTHROPOIETIN RECEPTOR EXPRESSED IN STEM CELLS ? Gregory Longmore, and Harvey Lodish., Whitehead Institute, Cambridge, MA, 02142.
Erythropoietin (Epo) is the major regulator of erythroid viability, proliferation and differentiation. The action of erythropoietin is restricted to early erythroid progenitors and possibly megakaryocytes. This is in contrast to other cytokines that affect the proliferation and differentiation of multiple hematopoietic lineages (IL-3, GM-CSF, IL-6). Cytokine signaling follows the binding of the cytokine to a specific receptor(s). The majority of hematopoietic cytokine receptors belong to the cytokine receptor superfamily. The receptor for erythropoietin (EpoR) is also a member of this family. The protein homology within this family of receptors resides in the exoplasmic domain. There is limited cytoplasmic region homology between a few but not all members of the family, and the mode of signal transduction by members of this receptor family is in large part unknown. We have isolated a mutated murine EpoR that is active in the absence of ligand. This constitutively activated EpoR (cEpoR) is oncogenic; inducing the development of erythroleukemia in mice infected with a Spleen Focus-forming Virus expressing cEpoR. cEpoR when expressed in the IL-3-dependent pro B cell line BaF3, the IL-2-dependent cytotoxic T cell line CTLL-2, and the Epo-dependent erythroleukemic cell line HCD57 abrogates the need for any added growth factor. These cell culture results suggest that cEpoR can supplant IL-3 and IL-2 implying that cEpoR when expressed in hematopoietic stem cells may exhibit pleiotrophic effects on hematopoiesis, as opposed to the restricted erythroid effect of the wild type EpoR. To test this hypothesis we have reconstituted lethally irradiated mice with bone marrow infected with a retrovirus expressing cEpoR. We have also generated embryonic stem (ES) cells expressing cEpoR. Transgenic mice derived from blastocyst injection with cEpoR-ES cells have been established. The results of these experiments will be presented.

M 420 CONTROL OF HUMAN FETAL GLOBIN GENE EXPRESSION DURING DIFFERENTIATION, Nadya, L. Lumelsky and Bernard, G. Forget*, Department of Biochemistry, University of Wisconsin, Madison, WI 53706 and * Department of Medicine, Yale University School of Medicine, New Haven, CT 06510
Under normal culture conditions, the human erythroleukemic cell line K562 expresses predominantly an erythroid phenotype, but undergoes a drastic change when exposed to tumor promoting phorbol esters. As a result the expession of a number of erythroid genes is extinguished and the expression of a number of megakaryocytic genes is initiated. Because it is believed that erythrocytes and megakaryocytes originate from a common progenitor, this system offers an excellent opportunity to examine the mechanisms of selectivity of gene expression between these two cell types. We found that the down-regulation of several erythroid proteins of the K562 cells takes place at the level of mRNA accumulation. Moreover, the most abundant erythroid protein of these cells, γ-globin, is down-regulated at both the transcriptional and post-transcriptional levels. We proposed that the post-transcriptional effect involves γ-globin mRNA destabilization. A gene transfer system using a marked γ-globin gene was employed to study the cis-acting regulatory elements that might mediate the effect of phorbol esters on the rate of the γ-globin mRNA turnover. The marked gene and its deletion mutant derivatives was stably introduced into K562 cells and its expression was compared to that of the endogenous γ-globin gene in the presence of phorbol esters. The results of these experiments will be shown.

M 421 GENERATION OF MONOCLONAL ANTIBODIES AGAINST ONCOGENE-TRANSFORMED AVIAN MULTIPOTENT/ ERYTHROID PROGENITOR CELLS, Kelly M. McNagny, Fabio Rossi, Sigrid Grieser and Thomas Graf, European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, D-6900 Heidelberg, Germany
E26 is a v-*myb*, v-*ets* containing avian leukemia virus capable of transforming early erythroid and myeloid lineage cells *in vitro* and *in vivo*. TPA treatment of clonal populations of phenotypically "early erythroid" cells results in the production of cells with either myeloid or eosinophilic morphology. A panel of four monoclonal antibodies was produced against these progenitor cells before and after TPA-induced differentiation. Two antibodies, MEP21 and MEP26, react with cell surface proteins, of 150 and 47-60 kD respectively, which are present on E26 transformed progenitor cells but whose expression becomes undetectable several days after TPA stimulation. In contrast a third antibody, EOS47, recognizes a 100 kD molecule expressed exclusively by a subset of E26 transformed cells after TPA stimulation but not by unstimulated progenitor cells. Within TPA treated cultures, the EOS47 is expressed by peroxidase positive cells (an enzyme which in avian species is only expressed in eosinophilic granulocytes) and a by subpopulation of peroxidase negative cells but not by cells expressing myeloid cell surface antigens. MEP21, MEP26 and EOS47 antibodies do not react with T, B, myeloid, or more mature erythroid lineage cell lines. The last antibody of the panel, MEP17, antibody reacts with a noncovalently linked heterodimeric molecule of 140 and 155 kD that is expressed at high levels by E26 transformed progenitor cells and at lower levels by TPA stimulated cells, and by cell lines representative of other hematopoietic lineages. The biochemical characteristics of this antigen suggest it is a member of the integrin family of adhesion molecules and may represent an avian homologue of the human VLA-4 antigen. All four of these antibodies react with minor subpopulations of cells in the bone marrow and spleen of chickens 1 day post-hatching. MEP21, MEP26 and EOS47 are not expressed by thymus or bursal cells, while MEP17 antigen is expressed by all bursal B cells. The distribution of these antigens on hematopoietic lineage cells suggest they may serve as valuable phenotypic markers for the delineation of early events in normal hematopoiesis and in leukemogenesis.

M 422 EXPRESSION OF BIOLOGICALLY ACTIVE HEMOPOIETIC GROWTH FACTORS IN THE FETAL GENITAL RIDGE, AND THEIR POSSIBLE ROLE IN PRIMORDIAL GERM CELL DEVELOPMENT.

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The biological activities of many recombinant cytokine molecules have been extensively characterized on cells of hemopoietic origin. It is now becoming clear that many cytokines, originally identified as hemopoietic regulatory molecules, may have additional roles in the development of non-hemopoietic cells. The mouse mutants White-spotting *(W) and Steel (Sl) both exhibit* defective hemopoiesis along with primordial germ cell (PGC) and melanocyte defects. The identification of the molecular nature of the mutation in Sl as stem cell factor (SCF) has led us to ask if other cytokine molecules, typically thought to be important for hemopoiesis, are expressed in the PGC microenvironment of the genital ridge. Primary or extended cultures of embryonic day 12 genital ridges spontaneously secrete IL-6 and M-CSF as measured by ELISA and specific biological assays. No expression was detected for IL-3, IL-4, IL-5, GM-CSF, LIF, TNFα, or IFNγ. mRNA for IL-6 and M-CSF was detected in freshly isolated genital ridge tissue, as was mRNA for the respective receptors, IL-6 receptor and c-fms. We have purified PGC from embryonic day 12 genital ridge to homogeneity by FACS, and are now testing these cells for responsiveness to SCF, IL-6, and M-CSF. Migratory PGC from the embryonic day 8 allantois are also being examined for responsiveness to SCF, IL-6, M-CSF and other cytokines. These results suggest a broader role in development for some cytokines, and also indicate similarities between PGC and hemopoietic precursor cells.

M 423 NUCLEAR HORMONE RECEPTORS RELATED TO THE RETINOIC ACID RECEPTOR IN THE MOLECULAR CONTROL OF HEMATOPOESIS.

Marianne Nussmeier, Bruce Blumberg, Steven Nimer and Edward DeRobertis, UCLA School of Medicine, Los Angeles CA 90024

The study of the molecular control of hematopoesis has to date chiefly emphasized cytokines and growth factors such as GM-CSF, Epo, and IL-3. In addition, families of DNA binding proteins act as transcription factors in the control of differentiation. For example, the retinoic acid receptor is involved in normal hematopoetic maturation *as well as myeloid leukemogenesis. We hypothesized that* a group of transcription factors with the highly conserved DNA binding domain seen in the retinoic acid receptor would be involved in the molecular control of hematopoesis, and would be expressed in the cells of the bone marrow.

A cDNA library was constructed from normal human bone marrow and screened with a degenerate oligonucleotide probe to detect all possible nucleotide sequences coding for the highly conserved DNA binding domain "CEGCKGF" of thyroid-retinoid receptors. Eight known members of this receptor family were identified: two retinoic acid receptors (RARα, RARγ), retinoid "X" receptor β (RXR β), an early response gene (TR 3), two peroxisome proliferator activated receptors (PPARα, PPARγ), human testis nuclear receptor (TR-2), and thyroid receptor α (ThyRα). In addition, two novel receptors designated as human orphan receptors (HOR 1, HOR 2), were found. We propose that this family of ligand dependent DNA binding proteins is involved in the molecular control of hematopoesis.

M 424 **CHARACTERIZATION OF C10, A NOVEL MEMBER OF THE MIP-1 CYTOKINE FAMILY,**

Amos Orlofsky, Mark S. Berger and Michael B. Prystowsky, University of Pennsylvania, Philadelphia, PA 19104

The mouse cDNA clone C10 was isolated by a differential screen designed to identify myeloid-specific clones in a bone marrow cDNA library. The cDNA sequence predicts a secreted protein of Mr = 10,749 after signal peptide cleavage. A protein of the expected size was observed in supernatants of C10-transfected COS cells. On the basis of amino acid sequence similarity, C10 belongs to the family of host defense-related cytokines of which the prototype is macrophage inflammatory protein-1 (MIP-1). The other members of this family are widely expressed upon activation of lymphocytes and/or macrophages. In contrast, C10 mRNA showed little or no response to activating agents and is constitutively expressed in only one of three macrophage cell lines, and two of four T-helper cell lines examined. This unusual regulatory pattern suggests that C10 may have a distinct functional role within the MIP-1 family. The results of functional tests of COS-derived C10 will be presented.

M 425 TRANSFERRIN RECEPTOR mRNA REGULATION DURING ERYTHROID DIFFERENTIATION,

Prem Ponka, Roxanne Y.Y. Chan and Herbert M. Schulman, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2

In proliferating non-erythroid cells the expression of transferrin receptors (TfR) is regulated in an inverse manner by the amount of intracellular iron. Fe-dependent regulation of TfRs occurs post-transcriptionally and is mediated by iron responsive elements (IREs) located in the 3' UTR of the TfR mRNA. IREs are recognized by a specific cytoplasmic binding protein (IRE-BP) that in the absence of Fe binds, with high affinity, to TfR mRNA preventing its degradation. While TfR numbers are positively correlated with proliferation in non-erythroid cells, in hemoglobin-synthesizing cells their numbers increase during differentiation and are, therefore, negatively correlated with proliferation. This suggests a distinct regulation of erythroid TfR expression and further evidence for this claim was found in the present study: 1) With nuclear run on assays our experiments show increased TfR mRNA transcription following murine erythroleukemia (MEL) cell treatment with DMSO. 2) DMSO treatment of MEL cells does not increase IRE-BP activity which is, however, increased in uninduced MEL cells by Fe chelators. 3) Following induction of MEL cells there is an increase in the stability of TfR mRNA whose level is only slightly affected by iron excess. 4) Heme synthesis inhibitors, such as succinylacetone (SA), which inhibit numerous aspects of erythroid differentiation also inhibit TfR mRNA expression in induced MEL cells. The possibility that receptor expression is inhibited by nonheme Fe accumulated as a consequence of SA-inhibited heme synthesis was ruled out by our results showing that the cellular ferritin content does not change. However, SA does not decrease TfR mRNA levels in uninduced MEL cells. These studies thus indicate that TfR gene expression is regulated differently in hemoglobin synthesizing as compared to uninduced MEL cells.

M 426 THE RESPONSE OF EARLY AND LATE HAEMATOPOIETIC PROGENITORS TO DIFFERENT 5-FU TREATMENTS AND ITS MODULATION BY CYTOKINE ADMINISTRATION,

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In vivo treatment with 5-Fluorouracil (5-FU) kills proliferating cells, which in bone marrow (BM) mostly comprise the "late" progenitors. Immature BM progenitor cells are subsequently recruited into cycle in order to replace the cell populations that have been eliminated. However, little is known about the effect of 5-FU on long-term reconstituting cells. In this study, the response of early repopulating stem cells, self-renewing uncommited progenitors and later progenitors was evaluated between 2 and 10 days after 5-FU treatment. The cycling status of these cells on day 5 was evaluated by their susceptibility to a second 5-FU injection. After in vivo treatment, BM was harvested and the content of haematopoietic progenitor cells determined ex vivo in assays detecting very primitive to mature precursors: long and short-term BM reconstitution, CFU-S, multiplication of preCFU-mix in suspension culture and colony assays.
We are currently investigating the effect of cytokine treatment on these responses. The results of in vivo treatment with a potential stem cell inhibitor, MIP-1α, in normal and 5-FU treated mice will be presented.

M 427 ABNORMAL SURFACE PHENOTYPE EXPRESSION IN AML,

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Cells from 214 AML patients were analyzed using 23 antibodies. Abnormal marker coexpression was found in 77% of all cases. Asynchronous expression of early and late *vs.* intermediate myeloid differentiation antigens (CD13-CD33+CD34-; CD13+CD33-CD34+; CD13-CD38+CD34-; CD13+CD38-CD34+) occurred in 30%, coexpression of T (CD2,5,7, or 25) with myeloid (CD13,14,15, or 33) in 29%, B (CD10,19,20, or 22) with myeloid in 8.5%, CD34 and late markers (CD15, CD11b, CD14 or CD56) in 17%, and CD33 with CD56 in 15% of the cases. Two- and three-color analyses confirmed coexpression in 5/5 cases, and indicated that these percentages are an underestimate, since coexpression can be demonstrated in cases without numerical overlap. The same phenotypes could be detected in minor populations of cells from remission patients. These data indicate that the aberrant coexpression of normal differentiation antigens is a common occurrence in AML, and that two- or three-color fluorescence analysis will not only aid in analysis of new AML patients, but also allow detection of the aberrant cells in remission. Rare/abnormal phenotypes were correlated with cytogenetics and with FAB classification. Significant correlations were discovered between t(8;21) cytogenetics and CD19 and CD15 or CD34 coexpression, t(9;22) and CD19 and CD34 coexpression, t(15;17) cytogenetics and coexpression of CD2 and myeloid antigens, diploid cytogenetics and coexpression of CD7 and myeloid markers, M5 FAB classification and coexpression of CD33 and CD56 and or the CD13-CD33+CD34- phenotype, M2 FAB classification and coexpression of CD19 and CD33, and CD7 and myeloid markers. CD33 and CD56 coexpression was significantly lower in M2 patients and CD7 and myeloid coexpression was absent in trisomy 8 patients. The presence of the CD13-CD33+CD34- phenotype was prognostic for a longer CR duration (p=.021), whereas the presence of the CD13+CD33-CD34+ phenotype was prognostic for a shorter CR duration (p=.001). Neither phenotype was prognostic for survival.

M 428 TUMOR NECROSIS FACTOR-α IS A POTENT STIMULATOR OF A VERY PRIMITIVE HEMATOPOIETIC STEM CELL IN LONG-TERM BONE MARROW CULTURES,

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We are using the long-term bone marrow culture (LTBMC) system of Dexter as a model to study the role of TNF in the regulation of hematopoiesis. TNF was added to fresh mycophenolic acid (MPA) treated cultures at various concentrations at the time of recharging. MPA treatment eliminates hematopoietic cells leaving an intact functional stromal layer which can then be reconstituted with stromally depleted bone marrow. After TNF treatment, the number of colony forming units-culture (CFU-C) in the cultures was greatly reduced by week 3 (post re-charging) while, at the same time, the number of colony forming units-spleen (CFU-S) was increased. This effect was consistent throughout the 5 week duration of the experiment. Supernatants from TNF treated and control cultures at week 2-5 were assayed for positive or negative factors on fresh marrow plated in soft agar and did not contain any activity that influenced colony formation. RNA extracted from TNF treated and untreated cultures and probed with cDNA's for macrophage colony stimulating factor (CSF-1), granulocyte-macrophage colony stimulating factor (gm-CSF), IL-3 or Il-6 showed no modulation in expression of any of those genes at the assay time points. Because fresh MPA treated stromal layers contain no colony forming activity in either soft agar or in day 12 CFU-S assays, TNF was added to MPA treated cultures not recharged with fresh marrow to determine the effect of TNF on purified stroma. TNF caused an immediate burst of hematopoiesis, with the first visible evidence of hematopoiesis appearing at day 2 post-MPA treatment followed by the appearance of assayable progenitors by day 7. Control MPA treated cultures showed no signs of hematopoiesis. This indicates that TNF stimulates a very primitive and quiescent progenitor spared by MPA treatment. These experiments are evidence that TNF is both a key positive regulator of a non-cycling hematopoietic stem cell and a negative regulator of committed progenitors in long-term cultures.

M 429 EFFECTS OF INTERLEUKIN-3 ON THE EPITHELIAL TURNOVER OF MURINE GUT MUCOSA,

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Colony stimulating factors (CSFs) such as IL-3, GM-CSF and G-CSF are currently being used in clinical trials to accelerate bone marrow recovery after chemotherapy or radiation therapy for patients with various tumors. A concern with the use of these cytokines is the risk that they may stimulate tumor growth. In particular, IL-3 and GM-CSF have been shown to stimulate the growth of pancreas and gastric carcinoma cells in vitro. We have been modelling in the mouse, a clinical study of the use of IL-3 for mobilization of stem cells from marrow to blood to facilitate their collection by apheresis. Because of the potential for IL-3 to stimulate the growth of epithelial tumors, we examined the effects of IL-3 on the epithelial turnover of murine gut epithelium. IL-3 (0.71 μgm/injection) was administered s.c. as two injections per day for 7 days. Controls received sterile distilled water. The animals were killed two hours after the last injection of IL-3. In addition to extensive hematopoietic evaluation, the entire gut was removed and segmentally divided into duodenum, jejunum, ileum and colon. Length and weight of individual segments was recorded. Mucosal pieces (1x1 cm) were removed from each segment and organ cultured with growth medium containing 1 μg/ml of vincristine sulfate. The cultures were terminated after two hours and the crypt microdissection technique was employed to determine crypt cell production rate (CCPR). There was no significant difference in the length and weight of various segments between IL-3 treated and control animals. However, there was a significant increase in the CCPR in all segments in the IL-3 treated animals compared to controls. Furthermore, the weight of ileal Peyer's patches was also significantly increased in IL-3 treated animals. These results suggest that the growth promoting properties of IL-3 are not restricted to hematopoietic cells when used in vivo and may directly or indirectly upregulate epithelial and lymphoid cell turnover in gut mucosa. (Supported in part by CA46686.)

M 430 IL-3 AND ERYTHROPOIETIN ENHANCE HEMATOPOIETIC PRECURSOR DEVELOPMENT DURING EMBRYONIC STEM CELL DIFFERENTIATION.
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We are studying the early events in hematopoiesis using in vitro differentiating embryonic stem (ES) cells as a model system. We have demonstrated that differentiating ES cells produce all of the in vitro colony forming units found in normal bone marrow, and were able to correlate the transcription of a number of cytokines and their receptors to the hematopoietic development which occurs during the differentiation of ES cells. One of the findings of these studies was that the IL-3 gene was not expressed at any time during the in vitro culture period. Since IL-3 is species specific, we concluded that IL-3 is not necessary for the development of hematopoietic precursors in this system, even though the IL-3-receptor appears to be expressed very early. Human cord serum substantially increases the number of in vitro colony precursors in the ES differentiation cultures compared to fetal calf serum. We have investigated whether any known hematopoietically relevant cytokines can substitute for the potentiating effect of human cord serum. Here we present data, that the combination of erythropoietin and IL-3 results in an equal or higher number of in vitro colony forming precursors than human cord serum. This shows, that the IL-3 receptor expressed by differentiating ES cells is functional, and that IL-3, although not required during the hematopoietic development of ES cells, is able (in combination with erythropoietin) to substitute for the hematopoiesis promoting activity in human cord serum.

M 431 JUXTAPOSED AMINO AND CARBOXYL TERMINAL DOMAINS OF HUMAN GAMMA INTERFERON: THEIR ROLE IN RECEPTOR-LIGAND INTERACTIONS, Gail F. Seelig and Winifred W. Prosise, Schering-Plough Research, U.S.A., Bloomfield, N.J. 07003

Using overlapping immobilized peptide octamers, the epitope of murine monoclonal antibody Mab 35 raised against human gamma interferon is identified to include amino terminal region 12-19 and carboxyl terminal region 132-139 suggesting the juxtaposition of these two domains in the native protein. Previously, we demonstrated that another Mab 47N3-6 raised against human gamma interferon also recognized amino acid segments near both the amino and carboxyl terminal regions of gamma interferon (Seelig et al., (1989) J. Int. Res. 9, 184). We describe peptides which were designed and synthesized to mimic a conformation of human gamma interferon which places the amino and carboxyl domains of gamma interferon in close proximity. Discontinuous peptide #1 (15-21-G-G-G-132-138) and discontinuous peptide #2 (15-29--111-118--130-138) contain the regions of the amino and carboxyl terminus of gamma interferon which are bridged by a glycine linkage or another potentially proximal region of the protein respectively. Each discontinuous peptide inhibits biological or receptor binding activities with an IC 50 of 15-50 uM. Whereas the peptide 131-137 alone appears to be much less effective in either assay requiring peptide concentrations of greater than 300 uM for significant inhibition. Rabbit polyclonal antibody (P616) raised against discontinuous peptide #1 recognized both linear peptide regions of 15-21 and 132-138. Polyclonal antibody P616 recognizes native gamma interferon on direct ELISA. While it also recognizes gamma interferon which has been treated with 0.5% SDS, it does so to a lesser extent suggesting that the three dimensional conformation of the native protein may contribute significantly to the epitope recognized. Polyclonal antibody P616, and monoclonal antibodies Mab 35 and Mab 47N3-6 each inhibit binding of 125-I labeled human gamma interferon to its cellular receptor on Daudi cells. Polyclonal antibody P616 also inhibited HLA/DR antigen induction by gamma interferon on Colo 205 cells. When used as immunogen, polyclonal antibody P616 generated an anti-idiotypic response which inhibited receptor binding on Daudi cells and which recognized soluble gamma interferon receptor on ELISA. The anti-idiotypic response suggests that regions at the amino and carboxyl terminus of human gamma interferon may play an important role in the interaction of this ligand with its receptor.

M 432 A GENETIC ANALYSIS OF GATA-1 IN ERYTHROID DEVELOPMENT *IN VIVO* AND *IN VITRO*. M. C. Simon*, L. Pevny#, F. Costantini#, and S. H. Orkin*. *Harvard Medical School and the Howard Hughes Medical Institute, Boston, MA 02115; #College of Physicians and Surgeons, Columbia University, New York, NY 10032
Transcription factor GATA-1 binds to GATA DNA motifs in the regulatory regions of globin and other red cell-specific genes, suggesting an important role in erythroid cell differentiation. To investigate the role of GATA-1 *in vivo*, we disrupted the X-linked GATA-1 gene by homologous recombination in a male (XY) mouse embryonic stem (ES) cell line (Pevny, Simon, Robertson, Klein, Tsai, D'Agati, Orkin, and Costantini; [1991] Nature 349: 257). Mutant ES cells contributed to all non-hematopoietic tissues analyzed, and to a white blood cell fraction, but failed to generate circulating, hemoglobinized red cells. These results demonstrate an essential function for GATA-1 in the production of definitive erythroid cells. To investigate the role of GATA-1 in primitive erythropoiesis in the yolk sac of a developing fetus, we employed an *in vitro* system that mimics embryonic hematopoiesis. When wild type and mutant ES cells were cultured *in vitro* in methylcellulose, they formed embryoid bodies that contained a variety of hematopoietic cells. Wild type ES cells produced visible erythrocytes after 8-10 days in culture and transcribed ζ-, ε-, and subsequently α- and β-globin mRNAs. However, embryoid bodies composed of GATA-1-deficient ES cells failed to produce visible red cells and globin transcripts. Therefore, the GATA-1 mutation blocks the production of primitive as well as definitive erythroid cells. Both wild type and mutant ES cells gave rise to macrophages and neutrophils *in vitro*. This confirms that other hematopoietic lineages are not affected by the mutation and that a pluripotent stem cell must not require GATA-1 for viability or development; multiple lineages would be ablated by the mutation if this were the case. The ability of mutant ES cells to produce erythrocytes *in vitro* is restored by the introduction of a 12 kb DNA fragment containing the GATA-1 gene. These "rescued" ES cells were also introduced into host blastocysts and shown to contribute to the red cell population in chimeric mice. The wild type and GATA-1-mutant ES cells provide a novel genetic system for studying the control of erythropoiesis *in vivo* and *in vitro*.

M 433 IDENTIFICATION OF NOVEL PROTEIN TYROSINE KINASES BY DEGENERATE PCR. Denise K. Simoneaux[1], Nguyen T. Van[2] and John W. Belmont[3,4], [1]Department of Microbiology and Immunology, [3]Institute for Molecular Genetics, [4]Howard Hughes Medical Institute, Baylor College of Medicine, and [2]Department of Hematology, M.D. Anderson Cancer Center, Houston TX 77030. In an attempt to identify novel receptor protein tyrosine kinases which may play a role in early hematopoietic development, we performed reverse-transcriptase polymerase chain reaction using degenerate primers corresponding to conserved amino acid domains in the catalytic region of the tyrosine kinase family. PCR was performed on total RNA from density separated mouse bone marrow; from lineage negative, low density, wheat germ agglutinin positive mouse bone marrow cells; and from two pre-T cell lines--2017 (Thy-1$^+$, CD4/CD$^-$) and 2052C (Thy-1$^-$, CD4/CD8$^-$). The expected 200 base pair PCR products were subcloned and analyzed by sequential rounds of sequencing and colony hybridization. Sequence analysis revealed the amplification of known tyrosine kinases: CSF-1 receptor, c-*fes*, Jak-1, Jak-2, and c-*abl*, *bek*, and tyro13. We have also identified two novel kinase genes. The first was identified in low density bone marrow cells and shares conserved amino acid sequence with the tyrosine kinase family including a conserved methionine in the consensus (K/T)W(T/M)APE which may be indicative of a receptor tyrosine kinase. Sequence comparison with known kinases indicate that this gene is most closely related to c-*met*. The second novel kinase gene was identified in the pre-T cell line 2052C. It also contains conserved amino acid sequence with the tyrosine kinases and appears most closely related to drosophila *src* 28. Data will be presented regarding unique features of these new kinase genes and their expression in hematopoietic stem cell enriched populations.

M 434 ERYTHROLEUKEMIA IN GATA-1/c-MYC TRANSGENIC MICE.
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To immortalize erythroid precursors we have targeted the expression of an proto-oncogene under the control of an erythroid specific promoter in transgenic mice. As the promoter we have chosen the regulatory elements of the mouse GATA-1 gene. GATA-1 gene expression has been shown to be restricted to cells of erythoid, mast cell and megakaryocytic lineages. The human c-MYC was used as the oncogene. We have generated 13 transgenic founders. Five founder animals developed rapidly progressive erythroleukemia within the first few weeks of life and all of them died between week 4-6. Two of the remaining 8 lines showed no expression of the transgene; the others are currently being investigated. Tumor cells from spleens were transplantable in nu/nu mice or syngeneic FVB and were clonogenic without addition of IL-3 in methylcellulose. Cell lines have been established from 4 out of 5 leukemic animals . These cells grow factor independently in suspension culture. They display proerythroblast morphology and constitutively express α and β globin and EPO-receptor. No terminal differentiation was observed in response to DMSO or EPO. The results show that c-MYC can transform erythroid precursors if expressed at a vulnerable phase and demonstrate the tissue specificity of the GATA-1 regulatory sequences used in this construct. Further characterization of the erythroid cell lines will be presented.

M 435 EFFECT OF VITAMIN A ON HUMAN PROGENITOR CELLS (CD34+ CELLS),
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We have examined the effects of vitamin A on positively selected CD34+ cells as well as on mononuclear cells from normal human bone marrow. Using physiological doses of retinol and retinoic acid in ethanol as well as the physiological carrier chylomicronremnant particles, we found that vitamin A in a dose-dependent manner specifically inhibited G-CSF-mediated growth of $CD34^+$ cells in liquid culture as well as colony-forming assays. No effect on differentiation was noted. When assessing the more immature progenitor cells in blast colony assays, we found that vitamin A in doses less than 100nM stimulated blast colony formation, whereas high doses (>1μM) were inhibitory. Taken together, the data indicate that vitamin A has different effects on progenitor cells at various stages of differentiation. The mechanisms involved in the vitamin A-mediated growth regulation is currently being investigated.

M 436 TNFα-mediated killing is regulated by C kinase,
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TNFα is a small receptor binding cytokine produced primarily by macrophages. TNFα was shown to induce rapid cell death after microinjection into L929 cells. This result suggests that receptors may serve as transporters as well as transducers of polypeptide hormones and that internalization of TNFα has important intracellular biochemical roles. Microinjection of TNFα (3 ng/ml) into serum starved NIH 3T3 cells induced two different responses (growth or death) depending on cell cycle. The ratio of growth induction to cell killing was dependent upon the length of time the cells were serum deprived. Starvation for ten hours results in 34% of the cells entering DNA synthesis while 66% die. Thirty hours of starvation resulted in 7% DNA synthesis induction and 93% killing. The cytotoxicity observed after injection of TNFα into NIH 3T3 cells was also increased if the cells achieved contact inhibition in addition to serum starvation. These data suggest that intracellular protection from TNFα cytotoxicity is linked to cell cycle regulated growth and that some factor may be involved in this protection which is degraded with time during serum starvation. Proliferation of fibroblasts often involves the C kinase-mediated phosphorylation of enzymes in metabolic pathways. To evaluate the role of C kinase in TNFα-mediated cytotoxicity, cells were treated with C kinase inhibitors or activators prior to TNFα injection. The cytotoxic effects of TNFα injection were enhanced by addition of C kinase inhibitors but blocked by PMA addition. Protection was also seen after direct co-injection with C kinase. The mechanism where by C kinase protects from the cytotoxic effects of TNFα is not understood. These studies suggest a mechanism for the pleiotropic activity of TNFα in different cell types and tissues: the response induced by TNFα may depend upon the activity of other regulatory molecules, such as C kinase, in each specific cell. Cells that do not utilize C kinase (perhaps to modulate the activity of a protective factor) or do not contain a protective protein would be more sensitive to the cytotoxic activity of TNFα.

M 437 PROTHYMOSIN α (PROTα) RNA LEVELS ARE RAPIDLY ALTERED IN DIFFERENTIATING HL-60 CELLS, BUT CORRELATE WITH PROLIFERATION, NOT DIFFERENTIATION.
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ProTα is expressed in a wide variety of cells, encodes a nuclear protein, and ProTα RNA levels increase when cells are stimulated to proliferate. To determine whether ProTα RNA is down-regulated during differentiation, HL-60 seeded at 3×10^4/ml or 1×10^5/ml were incubated with r-Hu-γ-IFN (Boehringer) at 200, 500 or 1000 U/ml, or with 16nM TPA or 1.25% DMSO. Total RNA was isolated at 1 and 3 days and ProTα and c-myc RNA assessed by Northern analysis. Cell growth was assessed by cell number and flow cytometric (FACS) cell cycle analysis after propidium iodide staining at 1,3 and 6 days. FACS analysis of cell surface MY4 (CD14) and OKM1 (CD11b) confirmed differentiation. Untreated HL-60 contain high levels of ProTα RNA. DMSO and TPA induce granulocyte or macrophage differentiation, respectively, and ProTα RNA levels significantly decrease by 4-6 hrs, a time course resembling that of c-myc regulation in this system. HL-60 cells treated with γ-interferon (IFN) undergo monocyte differentiation yet continue to divide. Under these conditions ProTα RNA levels do not decrease, demonstrating that ProTα RNA regulation correlates with proliferation, not differentiation. The table shows one experiment, day 3, 200U/ml γ-IFN. Similar results were observed at higher doses of γ-IFN and other times.

AGENT	(CELL #)	%S+G2/M	%CD14	%CD11b	PROTα	MYC
NONE	(3×10^4)	57	19	17	+	+
DMSO	(3×10^4)	31	15	56	-	-
TPA	(3×10^4)	30	24	95	-	-
γ-IFN	(3×10^4)	47	87	91	+	+
γ-IFN	(1×10^5)	52	93	94	+	+

When HL-60 differentiate but continue to divide both ProTα and c-myc RNA remain elevated, in contrast to down-regulation of both RNAs when cells terminally differentiate. This further supports the hypothesis that ProTα is a nuclear oncoprotein with a role in cell proliferation and differentiation.

M 438 MULTILINEAGE RESPONSE IN APLASTIC ANEMIA PATIENTS FOLLOWING LONG-TERM ADMINISTRATION OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR,

Yoshiaki Sonoda, Yoichiro Ohno, Hiroshi Fujii, Takayuki Takahashi, Hiroshi Hara, Akihisa Kanamaru, Eizo Kakishita, Atsushi Horiuchi, Tatsuo Abe, Haruto Uchino, and Kiyoyasu Nagai, The Department of Hygiene, Kyoto Prefectural University of Medicine, and The Aplastic Anemia Treatment Study Group, Kyoto 602, Japan.

Our preliminary study suggests that long-term administration of recombinant human granulocyte colony-stimulating factor(rhG-CSF) induced a bilineage response in refractory aplastic anemia(AA) patients(Sonoda Y et al. Eur J Haematol, in press). The present multicenter study was undertaken to confirm whether rhG-CSF would mobilize residual multipotential stem cells by its G_0 shortening effect in patients with AA and induce a multilineage response. 28 cases with acquired severe or moderate AA received long-term administration (2-12+ months) of rhG-CSF (provided by Kirin Brewery Co., Tokyo, Japan) in doses from 100-500μg/body/day by subcutaneous injection or 500-1,500μg/body/day by intravenous infusion. 23 out of 24 evaluable cases showed a substantial increase in neutrophils associated with a recovery of myeloid precursors in bone marrow within 1 month of therapy. Interestingly, 8 out of 24 cases showed a dramatic improvement in severe anemia after 2-4 months of therapy. Moreover, severe thrombocytopenia improved in 2 out of these 8 cases accompanying a significant increase of megakaryocytes in bone marrow. Clonal cultures revealed the recovery of myeloid as well as erythroid progenitors in these 8 cases. In 2 cases, who showed a trilineage response, mixed and megakaryocyte colony formations were also recovered. These results suggest that long-term administration of rhG-CSF mobilizes myeloid, erythroid, megakaryocyte, and multipotential progenitor cells and induces a multilineage response in some patients with AA.

M 439 THE IN VIVO BIOLOGICAL ACTIVITY OF MURINE IL-4 AND THE ROLE OF ENDOGENOUS CYTOKINES

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We employed alginate entrapped cells secreting recombinant murine IL-4 to study the effect of continuous dosing on the hematopoietic cells of normal mice.

The peritoneal cell populations showed an increase in monocytes and eosinophils with an enhanced phagocytic capacity. These increases were inhibited by anti IL-4 Mab but not isotype control Mab. Anti GM-CSF blocked the increase in monocytes and phagocytosis but not eosinophil cell number. Anti IL-3 had no effect on eosinophils or the increase in phagocytosis at day 9 but blocked the increase in monocytes and day 5 phagocytosis. Anti IL-5 had no effect on IL-4 induced phagocytosis or monocyte number but decreased the number of eosinophils. In the spleen and peripheral blood, IL-4 induced a 2 to 3-fold increase in T4, T8 and B220 positive lymphocytes. These effects were completely blocked by anti IL-4 Mab. In the peripheral blood, anti GM-CSF, IL-3 and IL-5 Mabs inhibited this effect on days 5 and to a greater extent on day 9. However, the increases seen in the spleen on day 9 were blocked >80% by the anti IL-3 and GM-CSF Mabs while anti IL-5 treated animals showed an increase in these cells. IL-4 induced a large increase in MAC-1 positive peripheral blood monocytes and granulocytes which was blocked by anti IL-4 and anti GM-CSF Mabs and partially blocked by anti IL-3 and IL-5 Mabs, most noticably at later time points. Similarly, IL-4 induced a large increase in splenic MAC-1 positive monocytes and granulocytes which was blocked 100% by anti IL-4 Mab and >80% by anti IL-3 and GM-CSF Mabs. Anti IL-5 Mab showed a slight effect on these populations.

M 440 ADMINISTRATION OF MONOCLONAL ANTIBODY TO IL-2 RECEPTOR β-CHAIN ABROGATES DEVELOPMENT OF THY-1^+ DENDRITIC EPIDERMAL CELLS,

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IL-2, a crucial growth factor for mature T cells, is produced in fetal thymus under the developmental control, although its biological significance remains unclear. The IL-2 receptor (IL-2R) is composed of at least two distinct subunits, IL-2Rα and IL-2Rβ. IL-2Rα has been shown to be expressed in a sizable fraction of murine fetal thymocytes, but expression of IL-2Rβ, a crucial element for IL-2 signaling, has been poorly characterized. We have produced anti-murine IL-2Rβ mAb TM-β1 (rat IgG_{2b}) that can effectively block IL-2 binding to IL-2Rβ and directly examined IL-2Rβ expression in fetal thymocytes. During day 14 - 19 of gestation IL-2Rβ was consistently detected in 2 - 4% of thymocytes. Expression of IL-2Rβ in fetal thymocytes was largely, if not entirely, restricted to $\gamma\delta$ TCR^+ cells, especially in $V_{\gamma}5^+$ cells. Furthermore, it was found that IL-2Rα and IL-2Rβ were expressed in an almost mutually exclusive fashion. The blockade of the IL-2/IL-2R pathway in the fetal stage by treatment with TM-β1 mAb resulted in the complete and selective disappearance of Thy-1^+ skin dendritic epidermal cells. Development of any other T cell subsets was normal. This result strongly indicates that a single lymphokine is selectively involved in development of a particular T cell subset in the embryo.

M 441 THE TRANSGENIC SAD-1 MOUSE EXHIBITS THE PATHOLOGIC FEATURES OF SICKLE CELL DISEASE,

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The SAD-1 transgenic mouse was generated by co-injection of the human α_2-globin gene and a modified β-globin gene linked to the β-globin locus control region (LCR). The β^{SAD} gene includes the β^{6VAL} substitution of human sickle cell disease (SCD) and two naturally occurring mutations which promote the polymerization of HbS, Antilles and D-Punjab. Red blood cells (RBC) of SAD-1 mice and double heterozygous β-thal/SAD-1 mice contain 19% and 26% HbSAD, respectively, and both sickle in vitro upon deoxygenation.

We performed histopathological analyses under ambient and low oxygen tensions to evaluate the SAD-1 transgenic mice as model for human SCD. Both at ambient oxygen tension and under hypoxia, RBC and late reticulocytes exhibit intracellular filaments identical in size and shape to those seen in human SCD. At ambient oxygen tension, the transgenic mice display features of steady-state human SCD including in vivo sickling, increased splenic and medullary erythropoiesis, congestive splenomegaly, hemosiderosis, glomerulosclerosis and occasional vascular thrombosis. The pathology is more severe in β-thal/SAD-1 mice. Under moderate hypoxia (pO_2 10%), the mice exhibit pulmonary microthrombosis and hepatic necrosis. More severe hypoxia (pO_2 8%) is fatal to βthal/SAD-1 mice within 60 minutes. At autopsy these mice display severe systemic vascular congestion and pulmonary hemorrhages, imitating human sickle cell crisis.

In conclusion, the SAD-1 mouse displays morphological and functional features which closely resemble those of human sickle cell disease and sickle cell crisis.

M 442 VLA-MEDIATED ADHERENCE OF NORMAL CD34+ CELLS AND MYELOID LEUKEMIC CELLS TO FIBRONECTIN IS ACTIVATION DEPENDENT.

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Direct interactions between hematopoietic cells and components of the bone marrow microenvironment play an important role in the regulation of hematopoiesis. Most of the presently known extracellular matrix receptors belong to the VLA-family. Little is known about the function of these antigens on normal and malignant human myeloid precursors. In the present study we analyzed the expression and function of VLA-antigens on purified bone marrow derived CD34+ cells and acute myeloid leukemic (AML) cells. Double labeling experiments (n=7) showed that normal CD34+cells express only VLA-4 (51%±28%) and VLA-5 (64%±28%) and no other VLA-antigens. Both antigens are also expressed on the cells of almost all tested 52 AML-patients (96% and 100%, respectively). VLA-4 and VLA-5 antigens were not homogeneously expressed on CD34+ cells. Cell-sorting and culture experiments with double-labeled CD34+cells showed that almost all CFU-GM were found in the weakly positive fractions for both antigens. In a solid phase adhesion assay, in which purified CD34+cells (depleted for CD19+CD34+ and CD36+CD34+ cells) were incubated in microtiter plates coated with fibronectin and albumin, it was shown that the VLA-antigens on these cells are in a non-functional state. This finding is in agreement with previously described results that myeloid precursors do not adhere to fibronectin. However, after activation of CD34+cells with PMA (10ng/ml) adherence to fibronectin could be demonstrated (see figure). This binding could be almost completely blocked by anti-VLA5 Moab. PMA activation did not influence the expression of VLA-antigens. Similar results were obtained with AML-cells.

% adherent CD34+
cells (mean of 5
experiments ±std)

M 443 *STEM CELL FACTOR (SCF) STIMULATES THE IN VITRO* GROWTH OF BONE MARROW CELLS FROM APLASTIC ANEMIA PATIENTS.

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Aplastic anemia (AA) is a rare bone marrow disorder manifested by a slow, or in severe cases, absent growth of hematopoietic precursors. The cause of the disease is unknown. As recently shown (Martin et al. *Cell* **603**, 203, 1990), *in vitro* proliferation of the hematopoietic progenitors can be augmented by a stem cell factor (SCF) produced by marrow stroma, a *c-kit* receptor ligand. We have attempted to rescue the growth of bone marrow cells from AA patients by culturing the cells in the presence of human recombinant SCF (provided by AMGEN). A significant increase of the bone marrow colony number has been observed with cells from 11 out of 15 AA patients studied. Cell proliferation, as measured by ^{3}H-thymidine incorporation, approached in some cases the level observed with normal bone marrow cells. The effect of SCF was up to 20 times stronger than effects of other hematopoietic growth factors, like IL-3, GM-CSF and G-CSF. Lack of response to SCF as observed in 4 patients suggests defects in the *c-kit* receptor or in its signalling pathway. These data indicate that abnormalities in *c-kit* function in marrow cells and/or in SCF expression by hematopoietic stromal cells may play a role in aplastic anemia.

M 444 The ß-globin LCR functions through GATA-1 and/or two novel factors which bind to the ß-globin gene promoter CAAT box, not through CP1.

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The human ß-globin locus control region (LCR) is an element that is required to express the ß-"like" globin genes at very high levels. The LCR acts erythoid-specifically and it isolates globin genes from effects of neighbouring genomic sequences. For the LCR to induce high levels of transcription from the promoter of the adult-stage-specific human ß-globin gene during differentiation of murine erythroleukemia (Mel) cells, either the CAAT box or CAAC box regions of the promoter must be present. Using gel-mobility-shift assays, we have shown that besides interacting strongly with the ubiquitous factor CP1, the CAAT region binds the erythrocyte-specific protein GATA-1 and two additional factors that have not been described previously. These two novel factors have a similar DNA-binding specificity and they also bind to the ß-globin CAAC region. Moreover, one of these factors was only detected in induced Mel cells. Thus, we have called this factor DSF-1 for differentiation specific factor, while the second is denoted DSF-r for DSF related.By mutating the CAAT box, in conjunction with stable transfection assays of Mel cells, we have found that the LCR can only produce high levels of transcription in induced Mel cells when either GATA-1 and/or the DSF factors bind to the CAAT region. Low levels of expression were obtained when only CP1 or none of the proteins could bind to a mutated CAAT box. These results suggest that the LCR acts directly through erythoid and/or differentiation-stage-specific factors, but not through ubiquitous factors. Moreover, binding sites for the DSF factors may only be present in the adult-stage-specific ß-globin gene. Thus, we propose that these factors may also be involved in the switching of expression from one to another globin gene during development. Supported by the National Cancer Institute of Canada and the Fonds en Recherche de Santé du Quebec.

M 445 OLIGOMERIZATION AND CONSTITUTIVE ACTIVATION OF THE ERYTHROPOIETIN RECEPTOR.

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Erythropoietin (EPO) is a serum glycoprotein hormone which is required for the survival, proliferation and differentiation of committed erythroid progenitor cells. Recently, a cDNA encoding the murine erythropoietin receptor (EPO-R) was isolated by expression cloning and found to have sequence homology with other cytokine receptors. Members of the cytokine receptor family share four conserved exoplasmic cysteine residues as well as a motif, Trp-Ser-X-Trp-Ser, which is located in the exoplasmic domain close to the membrane spanning region.

A constitutively active mutant of the EPO-R (cEPO-R) was isolated which was found to contain a single point mutation within the extracellular domain, converting residue 129 from an arginine to a cysteine. This activating point mutation enables the receptor to induce hormone independent growth after its introduction into the IL-3 dependent BA/F3 cell line. We investigated the function of cEPO-R using both biochemical and mutagenesis approaches. Our studies demonstrate that the novel cysteine at residue 129, rather than the loss of an arginine, in cEPO-R is required for constitutive activity. The constitutive receptor forms disulfide linked dimers, most likely through cysteine 129. These dimers are present on the plasma membrane and the presence of dimers in various mutants of the EPO-R is correlated with constitutive activity.

M 446 IN VITRO STIMULATION OF MYELOPOIESIS BY CONSTITUTIVE EXPRESSION OF BASIC FGF IN RETROVIRALLY TRANSDUCED NIH 3T3 CELLS, Robert Wieder[1], Sanjay Shirke[1], Efimia Kehagias[1], Ann Jakubowski[1], E. Lynette Wilson[2] and Janice L. Gabrilove[1], [1]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 and [2]Department of Cell Biology and Kaplan Cancer Center, New York University Medical Center, New York, NY 10016

A 1 kb human basic fibroblast growth factor (bFGF) cDNA was cloned along with constitutive CMV or human ADA promoters into N2, a Moloney leukemia virus-based vector. The constructs were packaged using cell lines GP+E-86 and GP+envAM12. NIH 3T3 cells were transduced with viral supernatants and selected in G418. Clonal lines were selected which contained bFGF levels from 0.04 ng to greater than 0.4 ng/10^6 cells. bFGF in cell lysates was measured by ELISA using rabbit anti human bFGF antiserum. High bFGF producers exhibited phenotypic transformation including growth in agar, loss of contact inhibition, decreased adhesion to culture plates and accelerated growth kinetics. Gamma irradiated high and low bFGF producers were seeded with CD34 antigen positive-enriched normal human bone marrow progenitors. After one day of cocultivation, the hematopoietic cells were assayed for colony formation by culture in semisolid agar with rhGM-CSF. Low levels of bFGF in the adherent cells correlated with a substantial increase in CFU-GM colony formation ($\geq$40 cells). At higher bFGF concentrations, colony formation was less efficient, and at the highest levels of bFGF production, colony formation was completely suppressed. Cluster formation was not significantly affected by bFGF content. These data correspond to studies which showed that low levels of exogenous bFGF stimulated myelopoiesis in vitro. We are also correlating the levels of bFGF with the expression of other genes.

M 447 THE REGULATION OF PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN BONE MARROW STROMAL CELLS, E. Lynette Wilson, Melanie-Jane Hannocks, Lisa Oliver, *Janice Gabrilove and Daniel B. Rifkin, Department of Cell Biology, NYU Medical Center, New York, NY 10016, and *Sloan- Kettering Cancer Center, New York, NY 10021.

We envision a proteolytic mechanism based on the generation of plasmin from plasminogen by plasminogen activators (PAs) in the release of various haemopoietic cytokines from the stromal cell layer. We have examined the effects of three cytokines relevant to haemopoiesis, interleukin-1 (IL-1), transforming growth factor ß (TGF-ß) and basic fibroblast growth factor (bFGF), on the production of PAs and their inhibitors (PAI-1 and PAI-2) by bone marrow stromal cells. Stromal cells secreted low basal levels of PA activity and significant amounts of PAI-1. The addition of IL-1 (10 - 10^4 Units/ml), bFGF (0.02 - 20 ng/ml) and TGF-ß (0.02 - 20 ng/ml) had profound stimulatory effects on secreted PAs. IL-1 stimulated enzyme secretion approximately 10 fold and the predominant enzyme species was tissue (t-)PA. Concomitantly, PAI-1 secretion was inhibited whereas that of PAI-2 was stimulated. bFGF stimulated PA activity but to a lesser extent than IL-1. The most profound stimulation of PA activity was obtained with TGF-ß, with increases of urokinase (u-PA) of greater than 50 fold with 0.2 ng TGF-ß/ml. PAI-1 was also elevated by this cytokine. These three cytokines are all relevant to bone marrow physiology. In all instances PAs and/or their inhibitors were modulated by physiological concentrations of these agents. We propose that plasminogen activation in the bone marrow regulates local growth factor release.

M 448 CHARACTERIZATION OF IL-11 RECEPTOR AND PROTEIN TYROSINE PHOSPHORYLATION INDUCED BY IL-11 IN MOUSE 3T3-L1 CELLS, T. Yin, K. Miyazawa and Y-C. Yang, Walther Oncology Center, Indiana University School of Medicine, Indianapolis, IN 46202

IL-11 is a multifunctional cytokine derived from bone marrow stromal cells. In addition to its ability to stimulate the proliferation of IL-6-dependent cell line, T1165, IL-11 significantly enhances the number of antigen specific antibody forming cells both in vivo and in vitro, augments IL-3 dependent megakaryocyte colony formation and stimulates hemopoiesis in both normal and immunosuppressed mice. Recent data have also shown that IL-11 can induce the synthesis of acute phase proteins and act as an adipogenesis inhibitory factor(1). To understand the molecular mechanisms involved in the multiple effector functions of IL-11, we have focused on the functional and biochemical characterization of IL-11 receptor (IL-11R) and examined the role of protein tyrosine phosphorylation in IL-11 mediated signal transduction in 3T3-L1 cells. The results showed that IL-11 strongly inhibited lipoprotein lipase (LPL) activity in 3T3-L1 cells and the suppression of LPL activity by IL-11 was controlled at the post-transcriptional level. The ability of IL-11 to inhibit LPL activity therefore reflected the expression of functional IL-11R on the cell surface. Scatchard plot analysis revealed the existence of a single class of high affinity IL-11R with a Kd of 3.49 x 10^{-10} M and a receptor density of 5140 sites/cell on 3T3-L1 cells. Affinity cross-linking studies with ^{125}I-IL-11 indicated that IL-11R consists of a single polypeptide chain of 151 KDa in size. Furthermore, IL-11 rapidly and transiently stimulated tyrosine phosphorylation of 152, 94, 47, and 44 KDa proteins. Induction of tyrosine phosphorylation by IL-11 was inhibited by tyrosine kinase inhibitor genistein but not by depletion of protein kinase C by long-term (48 h) TPA treatment, suggesting that IL-11 specifically activates protein tyrosine kinases . These results demonstrate that IL-11 is closely linked to a functional protein tyrosine kinase pathway and tyrosine phosphorylation may be a key step in the initiation of the IL-11 receptor mediated transmembrane signaling. (1) I. Kawashima, et al. (1991) FEBS Letter 283:199-202.

Late Abstracts

NEUTROPHIL ACTIVATING PEPTIDE 2 (NAP-2) BINDS WITH TWO AFFINITIES TO HUMAN NEUTROPHILS,

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NAP-1 and NAP-2 are potent chemoattractants and activators of granulocytes with a wide range of proinflammatory effects (1). We studied receptors of NAP-1 and NAP-2 on human neutrophils by competition binding experiments and found that NAP-1 bound with one affinity, NAP-2 with two quite different affinities, to common receptors on neutrophils (2). Two affinities for NAP-2 also became apparent in binding kinetic experiments by measuring association and dissociation rates separately, and in chemical cross-linking experiments. A model for NAP-1/NAP-2 binding to their receptors on neutrophils will be discussed.

(1) Oppenheim, J.J., et al. (1991), Ann. Rev. Immunol. 9, 617.
(2) Schnitzel, W., et al. (1991), Biochem. Biophys. Res. Comm. 180, 301.

EMBRYONIC STEM CELL-DERIVED HAEMATOPOIESIS

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Embryonic stem cells can give rise to several lineages of mature haematopoietic cells when cultured under specific conditions (Doeschman *et al*, 1985; Wiles & Keller, 1991). In addition, we have shown that the entire haematopoietic system of lethally irradiated mice can be reconstituted longterm with ES-derived cells using the foetal liver of completely ES-derived embryos (Forrester *et al*, 1991). These embryos are generated by the aggregation of ES cells with developmentally-compromised tetraploid embryos. Because ES cells can be genetically manipulated *in vitro*, these approaches make it possible to analyse the consequences of introducing specific genes on haematopoietic cell proliferation and differentiation without the need for germline transmission.

We are using these approaches to study the role of the receptor tyrosine kinase encoded by the c-*kit* gene, and its ligand, Steel factor, on the differentiation of haematopoietic cells. We have generated ES cell lines that overexpress c-*kit* and are in the process of testing their *in vitro* differentiation potential in the presence of Steel factor. We have also generated ES cell lines that overexpress *Fli-1* (Friend leukemia integration 1), a gene that was indentified as a highly preferred integration site for Friend murine leukemia virus (F-MuLV), to assess its role in normal erythropoiesis.

Doetschman, T.C. et al. (1985). *J. Embryol. exp. Morph.* **87**, 27-45.
Wiles, M.V. & Keller, G. (1991). *Development* **111**, 259-267.
Forrester, L.M. et al. (1991). *Proc. Natl. Acad. Sci.* **88**,7514-7517.

PROTO-ONCOGENES MYC/MYN AND MYB: NEGATIVE REGULATORS OF MYELOID DIFFERENTIATION AND ROLE IN LEUKEMOGENICITY.

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Since blocks in differentiation play a role in leukemogenesis, it is important to dissect the genetic program of myeloid differentiation. We have employed the approach of manipulating the expression of genes which exert pleiotropic effects on gene expression to ascertain the effect on growth and differentiation of both normal and leukemic myeloblasts. Using recently established transfectants of the murine myeloid leukemic M1 cell line which constitutively express the proto-oncogene c-myb (M1myb), we have shown that enforced expression of c-myb blocks IL-6 or LIF induced differentiation at the myeloblast stage. Induction of the differentiation primary response genes (MyD) takes place; however, unlike in IL6/LIF treated M1 cells, they are subsequently suppressed in M1myb cells. C-myb expression blocks loss in leukemogenicity induced by IL-6/LIF in M1 cells, consistent with the notion that blocks in differentiation play a role in tumorigenicity. Enforced myb expression blocks differentiation at an earlier stage in the developmental program than enforced expression of the proto-oncogene c-myc (Mol.Cell.Bio. 11:2375-2381, 1991). In addition, we have shown that the proto-oncogene c-myc and its molecular partner myn are negative regulators of myeloid differentiation of both normal and leukemic myeloblasts. Evidence for how the loss of myc and myn function activates the differentiation program, bypassing cell surface events, will be presented.

ZINC-FINGER AND LEUCINE-ZIPPER TRANSCRIPTION FACTORS AS POSITIVE REGULATORS OF MYELOID DIFFERENTIATION.

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To better understand the molecular mechanisms and identify the genes that play a role in the regulation of hematopoietic cell differentiation, we have constructed cyclohexamide (CX) cDNA libraries which were differentially screened to isolate cDNA clones of myeloid differentiation primary response (MyD) genes, activated in the absence of protein synthesis, in HL60 and M1 cells following induction for macrophage or granulocyte differentiation. In the course of this work the zinc-finger transcription factor Krox24 has been identified as a myeloid differerentiation primary response gene, specifically induced upon HL60 and M1 macrophage differentiation. Also, the leucine-zipper transcription factors C-jun, junB, and junD were identified as myeloid differentiation primary response genes stably induced upon M1 terminal differentiation (Lord et al., MCB 11:4371,1991). Intriguingly, c-fos, although stably induced to high levels during normal myelopoiesis, was not among the MyD genes induced upon M1 differentiation. To gain further insights into the role of Krox24 and fos/jun transcription factors during normal myelopoiesis and in leukemogenesis, we have used differentiation inducible myeloid leukemia cell lines and normal primary myeloblasts, in conjuction with antisense oligodeoxynucleotide in the culture meadia and DNA transfer methodologies. Using these approaches, evidence will be presented to indicate that Krox24 and fos/jun transcription factors are positive regulators of myeloid differentiation.